

nature**15 November 1979**

Last chance for UNCSTD?

THERE have been two main reactions to the outcome of the United Nations Conference on Science and Technology for Development, which took place in Vienna in August. Some delegations returned home convinced that the conference had been a success; they support this claim by emphasising the amount of common ground established between developed and developing countries, and the general agreement on directions for future cooperation. Others, perhaps greater in number, returned feeling the conference had been a failure — but a qualified one. It had not resulted in any major shift in research towards Third World needs, or any particular clarification of what these needs are — but a framework had been agreed for pursuing both of these objectives.

Which of these two interpretations eventually prevails will depend on the outcome of the debates scheduled to take place next week at the UN General Assembly in New York. The Assembly's second committee will be discussing both the results of the Vienna conference, and the specific ways in which actions agreed by the conference should be carried out. Unfortunately many points of contention still remain, and it would be a tragedy if the short-term perspective engendered by the economic problems of the developed countries, as well as continued in-fighting within the UN system over future roles and responsibilities, placed in jeopardy the fragile achievements of Vienna.

There has already been some progress since the conference ended. The Director General for Development and International Economic Cooperation has prepared a detailed outline for the new intergovernmental committee (IGC) to oversee 'science, technology and development' issues. The Advisory Committee on the Application of Science and Technology to Development (ACAST), which despite recent criticism has considerable past achievement to its name, will be absorbed into a new body to advise the IGC. And the United Nations Development Programme, charged with administering the interim fund which the conference agreed should be set up on voluntary contributions totalling at least \$250 million for 1980 and 1981, has produced a business-like prospectus outlining how it will carry out this role.

But it would be wrong to think that next week's meeting will be anything like plain sailing. There are several controversial issues still to be decided, in particular the division of responsibilities within the UN structure for science and technology activities, and levels of appointment and staffing to service the new committee.

The Group of 77, which has been negotiating for the developing countries, is expected to present its proposals to the second committee outlining its views on these matters. And given their importance to both developed and developing countries — the Vienna conference, for example, was unable to agree on the specific role which the IGC should play with respect to the interim fund — heated discussion seems inevitable.

In terms of extra financial transfers, the situation looks even bleaker than it did in Vienna. Britain has just announced new foreign aid cuts which make it even more unlikely that it will be prepared to contribute to the interim fund when a pledging conference is held next February. The French position on an apparent commitment to increased UNDP contributions remains ambiguous. And Canada is reported to be having second thoughts about its proposal to shift funds within its aid budget to support joint research projects between Canadian and Third World research workers: Canada's International Development Research Centre, which had been expected to receive some of the new funds, is having its expansion plans closely scrutinised.

Perhaps the most alarming news comes from the United States, where the new Institute for Scientific and Technical Cooperation proposed by President Carter and offered as a centrepiece of the US presentation at Vienna is in serious difficulties in Congress. The Senate has refused to provide any funds for the institute's operation. Despite pressure from the House of Representatives, legislators seem determined to turn the ISTC into a sacrificial lamb on the altar of reduced public spending and federal bureaucracy. And its fortunes do not augur well for Congressional response to the \$25 million request which President Carter is expected to make in January for the UNDP interim fund — already the source of considerable controversy in the State Department — over where the money is to come from.

Yet if there was one message to come through from UNCSTD, it was that helping to promote scientific and technological activities in the Third World is not a question of handing out blank welfare cheques — it is an essential prerequisite for a flourishing global economy.

The tighter the purse strings, the more difficult this will be to achieve. And ironically the more important it becomes to ensure that international mechanisms such as the UNDP and the new IGC can be made to work fairly and effectively. □



US scientists double estimates of ozone depletion rate

A COMMITTEE of the US National Academy of Sciences has calculated that the worldwide use of halocarbons may be depleting the ozone content of the stratosphere twice as fast as was predicted three years ago. A report published by the academy in 1976, calculating a depletion rate leading to an eventual 7.5% reduction in ozone, became the basis for a ban in the US, implemented at the end of last year, on all non-essential uses of chlorofluorocarbons, particularly those used as aerosol propellants.

Using improved computer models and better atmospheric and laboratory measurements, the panel on stratospheric chemistry and transport of the Academy's National Research Council now estimates the most probable eventual depletion as 16.5%.

Furthermore, using a model that assumes "an increasing release rate in other industrial countries" — rather than a release rate fixed at the 1977 level, the panel says it is "highly probable" that the eventual steady state reached would

involve a 30% ozone reduction — and that this could be as high as 56.7%.

The panel points out that many uncertainties still surround such calculations. Since the release of earlier reports indicating the possible danger from fluorocarbon use, however, it points out that there has been a considerable improvement in both computer models and the quality of atmospheric measurements used to check their validity.

These activities have not altered the principal conclusion reached in the previous reports that continued release of halocarbons into the atmosphere will result in a significant decrease in the amount of stratospheric ozone," the report says.

Based on continued emission at the 1977 level the report says its own best estimate of the eventual ozone depletion is 16.5%. It adds that its estimate of uncertainty is such that there is a 95% probability that the true value of the ozone depletion lies between 5% and 28%. Ozone reduction to half the eventual level — to 8% — would occur in about 30 years, the report adds. □

Herbicide claimed responsible for birth defects

A US chemical trade union claimed last week that the exposure of male workers during production of the herbicide Oryzalin may have resulted in genetic damage to the children that they subsequently conceived.

According to the International Chemical Workers Union, of the four children born to men working in the plant in Rensselaer, New York State, over the period January 1975 to June 1976 during which the herbicide was being produced, three were born with heart deformities — two later dying — and one died from pneumonia two months later. There was also one miscarriage.

Oryzalin is the trade name for the chemical 3,5,-dinitro-N(4), N(4)-dipropylsulfanilamide. It is marketed in the US by Eli Lilly and co, which has denied that there is any evidence connecting the worker's exposure to the birth defects.

The company points out that no birth defects were indicated in laboratory tests carried out with rats and rabbits before the herbicide was approved by the Environmental Protection Agency in 1975. Nor was any evidence discovered after the question of a link to the human birth defects first raised in 1976. However, the assistant administrator of the EPA, Mr Steven Jellinek, said that the Agency was "very concerned" about the union's charges. Although the EPA did not know of any "scientifically established link" between exposure to Oryzalin and these

health problems, the union's information indicated that there might well be some hazard, either from the herbicide or from some other substance to which the workers were exposed.

The union has asked the federal government to ban the production and use of Oryzalin, which is used on soybeans for weed control. "We are taking these actions to alert the public to the potentially devastating effects to future generations from exposure to this chemical" said union President Frank D Martino.

Mr Jellinek said that although no problem had shown up in the animal tests, the human experience indicating possible genetic problems with workers exposed to the herbicide "does raise questions". He said that the EPA will set up a team with the Occupational Safety and Health Administration and the National Institute of Occupational Safety and Health to study the effects of the herbicide, and that the report should be ready "within weeks".

Last week the EPA suspended all use of the pesticide DBCP while it decides whether to impose a permanent ban on the chemical, which has been linked both to cancer, and to sterility in male workers involved with its production. The administrator of the EPA, Mr Doug Costle, has ordered all applications of DBCP — most commonly used on fruit trees and soybeans — to be halted except in Hawaiian pineapple groves.

David Dickson

US sifts ex-Nazi scientists for possible war criminals

THE US Department of Justice, stepping up its search for Nazi war criminals who may now be resident in the United States, is investigating whether any were included in the 500 scientists and engineers who were brought over from Germany at the end of the second World War to work on weapons research.

No details have been given of the names of individuals under investigation. However, Justice Department officials confirmed last week that some of the scientists and engineers involved in what was known as "operation paperclip" are among the department's list of 200 to 250 suspected war criminals.

Under the programme, which operated from 1945 to 1955, over 3000 German and Austrian scientists who had worked for the Nazis were checked by US authorities to discover whether their skills could be of value to the US military efforts — and thus might also be of value to the Soviet Union.

Over 500 were subsequently brought to the US under a programme approved by President Truman, despite strong opposition from scientists such as Albert Einstein.

Since it was felt that it was in the interests of national security to bring them to the US, the scientists and engineers were admitted without having to pass the usual immigration and visa checks, even though some were known to have been members of the Storm Troopers, and the League of National Socialist German Physicians.

Perhaps the best known of those admitted were the members of the team which worked on guided missiles under Dr Werner von Braun, and were largely responsible for launching the first US satellite in 1958. However, the US also brought over experts from a variety of other disciplines, such as three scientists who had worked on the German chemical weapons programme, who went to work for the Army's Chemical Warfare Division.

Mr Martin Mendelsohn, deputy director of the Justice Department's newly-created office to investigate Nazi war criminals, said last week that membership of a Nazi organisation was not necessarily sufficient to invoke charges, which required evidence of war crimes. He confirmed that his office is now examining the files of all those brought to the US under operation paperclip to see if any cases should be pursued further.

A typical case demanding attention, according to a staff member of the Senate committee which is pushing the Administration for action on war criminals, is that of a former concentration camp medical officer who came to the US to work on a variety of aviation medicine projects. □

Rasmussen vindicated?

The Three Mile Island accident might have been prevented if more attention had been paid to a report on nuclear risk, but its message was clouded by disputes. **David Dickson** reports

ONE unexpected spin-off from the accident at the Three Mile Island nuclear plant last March has been a shift in attitude towards a report on the safety of nuclear reactors published in 1975 — the much-criticised Rasmussen Report.

Known officially as Wash 1400, and commissioned by the Nuclear Regulatory Commission, the report is usually referred to under the name of its principal author Dr Norman Rasmussen, now professor of nuclear engineering at the Massachusetts Institute of Technology.

When the report first appeared, the nuclear industry made much of the report's conclusion that the level of risk associated with nuclear power was relatively low compared to other energy sources. But it also received a barrage of criticism, in particular over the validity of broad conclusions drawn from limited data.

Much of this criticism was subsequently endorsed by a review committee established by the NRC, which repudiated some key sections. But the report of the President's Commission on the TMI accident, chaired by Dr John Kemeny and released two weeks ago, points out that the sequence of events leading to the accident supports Rasmussen's general analysis — with the implication that if more attention had been paid to the positive aspects of the report, the accident might have been avoided.

Professor Rasmussen is unapologetic about the report. He accepts that some aspects of the accident, in particular the extent of its psychological impact, were not predicted. But he points out that the report emphasises the dangers of concentrating safety planning on single major equipment failures, rather than on an accumulation of relatively minor failures compounded by operator error. "If anything, we were too conservative. We said that if the temperatures in the core went as high as they did, then we did not know if it would melt or not, but said it might have done. In the event the core did not melt, though a few more human errors would have caused it to do so."

Part of the problem is that there are, in a sense, two Rasmussen reports. The first is a detailed application of fault-tree/event-tree analysis to equipment failures in nuclear power plants. Even critics acknowledge this as an important contribution towards understanding risks. And the NRC review, carried out by a committee under Professor H W Lewis of the University of California, stated that the methodology developed "both can and should be more widely used by the NRC".

The second version of the report, however, is the one that was presented through the news media as carrying the

principal message that nuclear power is basically safe. This conclusion, for example, helped persuade Congress to accept the Price-Anderson Act, limiting the liability of power companies in the case of nuclear accidents.

Professor Rasmussen is himself critical of those who over-sell the safety of nuclear power. "In the past there was a general tendency of public relations people to under-estimate the intelligence of the public, leading to the feeling that it was sufficient to issue warm pacifying statements rather than realistically portraying the risks and benefits of nuclear power. As the facts have evolved, however, the nuclear supporters have lost credibility, and many of their arguments are no longer believed."

Credibility has been at the heart of arguments over the Rasmussen Report itself. The Lewis Committee, following the lead of groups such as the Union of Concerned Scientists, criticised in particular the error bounds placed on the probabilities of various accident sequences.

They argued that these bounds were greatly understated, partly because of an inadequate data base, and partly because of an inability to quantify "common cause" failures, such as fire and earthquakes.

Professor Rasmussen defends his judgment. "I disagree with the Lewis Committee about margins of error. They found that the uncertainty was understated. I say that it might be understated in the safe direction, but I do not think it was greatly understated in the unsafe direction, because we already have 600 plant years of operating experience, and we have not melted a core. This gives an upper bound which is 30 times higher than ours".

It was the debates over the limits of uncertainty that led to the widest public criticism of the report. The NRC, for example, earlier this year issued a statement emphasising that as a result of its review "It does not regard as reliable the reactor safety study's numerical estimate of the overall risk of reactor accident."

Ironically, the vulnerability of the probability analysis seems to have resulted in that part of the report which was supported by the Lewis Review not getting the attention that it deserved.

A report prepared by the technical staff of the Kemeny Commission says that Wash 1400 contained three important messages. These involved the expected frequency of accidents (the TMI accident was well within the predicted range), methods for improving reactor safety through, for example, the use of fault-tree analysis to identify weak links, and the most likely types of accidents.



Rasmussen: unapologetic about the report

"Perhaps it is a fault of the report that these messages were not emphasised, because the conclusion most often associated with Wash 1400 — reactors are safe — receives the primary emphasis in the report. Perhaps it was the fault of the NRC that more effort was dedicated to criticising Wash 1400 than was applied to understanding its messages," the technical report (which does not necessarily reflect the Commission's views) says.

Rasmussen's critics argue that the construction of the report made it directly vulnerable to lop-sided interpretation. According to Dr Frank von Hippel of Princeton University, many scientists who read the executive summary of the report — also criticised by the Lewis Committee — "were infuriated by its Madison Avenue tone."

"If you follow the report backwards, from the appendices to the report itself to the executive summary, you find that at each level the qualifications and uncertainties of the previous level are dropped, so that what comes out is misleading because of what is omitted," he says.

Professor Rasmussen accepts some of the Lewis criticisms — but repeats that the TMI accident underlines the report's main conclusions. "The anti-nuclear people were able to use the accident to their advantage. But you can draw some satisfaction for both sides. Utility operators are now more prepared to listen to suggestions about safety than they were before, and on both the regulatory and the operating side, the event will lead to substantially reduced risk."

The accident had done nothing to change his support for nuclear power, a position now frequently expressed on public platforms. "Every new technology, when it was poorly understood, has attracted its doomsday prophets. Mostly this opposition has died away, and I suspect that nuclear power will probably go the same way. If we do not go on building nuclear plants, we will soon have major problems with the reliability of our power supply." □

Swedish firm in recombinant DNA storm

RECOMBINANT-DNA research in private firms in Sweden has got off to a very shaky start. The only firm to have applied to use the technique is threatening to move abroad if permission to go ahead is not given.

The firm concerned, KabiGen AB, is half owned by the state pharmaceutical company Kabi Vitrum, and half by private interests. It was set up in 1968 to investigate new sources of raw materials for Kabi Vitrum and is currently researching the production of human growth hormone by recombinant DNA techniques. Recently, the firm applied to the Hybrid-DNA Committee for permission to go ahead with further development. Permission was given to carry out the work P3/EK1 or P2/EK2 conditions under the revised NIH guidelines.

Kabigen already had a virology laboratory nominally between P3 and P4 standards; but as there were small parts of it which could collect dust, the firm announced that it would rebuild it. But an article in a Stockholm daily paper, written by the chairman of the Centre Party organisation in the same area as KabiGen, was very critical of the firm's plans to build the laboratory, especially as it is in a densely-populated part of Stockholm, and demanded that nothing be done until the whole issue had been debated publicly.

KabiGen's director, Dr Bertil Aberg, said that the firm's existence hinged on the laboratory being built and that the research would be moved abroad if the local health and building authorities did not approve the proposed changes and give permission for recombinant-DNA work to be done there.

The Centre Party — the carrier of the

Swedish anti-nuclear banner — drew a parallel between the two issues, saying that unless there is a public debate now, recombinant-DNA research would become the nuclear debate of the 1980s. But the Party had not checked its facts. While claiming that KabiGen was proposing to build a "high risk laboratory . . . without counterpart in Sweden", it was evidently unaware that there are already 25 P3 laboratories within a five-mile radius of Stockholm, handling dangerous pathogens in the State Bacteriological Institute and in local hospitals. It is doubtful, too, whether making minor alterations in the existing laboratory could be called "building". In spite of this, Dr Aberg seems to regret his initial openness. "KabiGen is the only industry using recombinant-DNA techniques", he told *Nature*. "Universities are doing a great deal of work with them, but they are very wisely keeping quiet about it."

However, this is not strictly true. All universities and private industries have agreed to inform the Hybrid-DNA Committee, set up in 1975 under the Natural Sciences Research Council, of any recombinant-DNA research they want to do. The committee then advises them about security levels. However this system will shortly be changed, if a bill proposed by the previous Liberal government is passed by the new Liberal-Centre-Conservative coalition. The bill is based on a one-man investigation of whether recombinant-DNA research can be carried out under Sweden's present laws. It proposed that a new body, to be called the Biotechnical Committee, be set up under the present Labour Protection Board, to advise the Board whether specific

experiments may be done and under what conditions. It would also follow international events in the field and keep the Board, government and public informed of developments.

According to the Bill, the committee should be composed of specialists in genetics, microbiology, biochemistry and plant breeding; representatives of the Social Welfare Board, Nature Protection Agency, relevant research councils, trades unions and employers' federations. The chairman of the committee should be the Director of the Labour Protection Board. The bill does not specify exactly how many members the Committee should have; but the report on which it is based suggested a composition which put the scientists in the minority. Until a decision on the new system can be taken, there is a moratorium on new recombinant-DNA work.

Predictably, many members of the Present Hybrid-DNA Committee (which has eleven members: seven scientists, one research engineer, one laboratory assistant and two Members of Parliament) are very critical of the proposed system. Professor Peter Reichard, the Chairman of the present committee, told *Nature* cautiously that "the new system won't be better than the present one." Other scientists were more forthright. "The new system would be enormously dangerous", declared Professor Lennart Philipson of Uppsala University. "It would be very bureaucratic: totally impossible. How much should you let public opinion aroused by newspapers influence the work of governmental agencies? Everything will depend on the people on the committee. How much common sense will they have?"

Wendy Barnaby

Avowed Nazis attack Brazilian physicists

A group calling itself the Movement for Nazi Renovation (MRN) has physically attacked scientists and intellectuals in Brazil. In a letter sent to the press they "take the responsibility for the terrorist attack upon the house of Mario Schemberg and his wife Lourdes Cedran". Schemberg is President of the Brazilian Physical Society; his wife was beaten up last month at her home by two men. At the same time several other Jewish physicists and artists received anonymous telephone calls.

The MRN criticised these people, some "for having international prestige, for being Jewish and as such diabolic", and others "for being living filth and all against the nuclear agreement" (between Brazil and Germany). The MRN also declared itself "against the feminist movement which, although pure nonsense, stimulates women to disobey men and have ideas of their own." The letter proclaims that MRN is "in favor of the nuclear agreement and of the Aryan race reassuming its historical

role, specially in the military domain."

"The situation is quite serious", Professor Jacques Danon of the Brazilian Centre for Physics Research in Rio declared in a manifesto signed by all members of the centre: "A professor of sociology from Campinas was abducted last week and forced to drink castor oil . . . What is most worrisome is that the government is trying to minimize these terrorist attacks."

The head of the Security Police of Sao Paulo declared that one should not be concerned about "a joke done in bad taste", while the influential ex-minister of education Passarinho declared that the whole "affair" was unimportant.

The scientific community is apprehensive. The end of formal political repression had indeed led to open criticism of Brazil's gigantic nuclear agreement with West Germany which involves the construction of eight 1000 MW nuclear power stations in the next five years

(*Nature* 2 August 79). The physicists presently under attack have all been particularly vehement in their opposition to the government's nuclear option for Brazil.

Recently Professor Cerqueira Leite said that the president of Nuclearbras, the government company which administers the nuclear contract, had gone from being a "nuclearcrat" to being a "nuclearpath" in accusing the physicists of participating in an international conspiracy against Brazilian interests allegedly involving both the United States and the Soviet Union.

The scientists are demanding in their manifesto that the authorities take the situation seriously. In the words of professor Danon, "even if MRN consists of a few crazy individuals, the government must prove that it has the will to act against them; otherwise some of our colleagues will be found dead tomorrow."

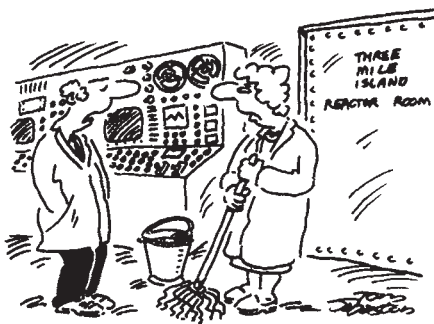
Maurice Bazin

news in brief

Research funds sought for Three Mile Island clean-up:

The owners of Three Mile Island nuclear plant said last week that they were negotiating with the Department of Energy over possible federal support for the Clean-up following last March's accident

as a research project, on the grounds that the work will be "a convenient way of learning more about reactor safety". Mr Herman Dieckamp, President of the General Public Utilities Corporation, told a Senate Committee that the DOE was planning to fund research that could be conducted either at the TMI plant; or on materials from the site for which the government would pay the utility company access fees and materials costs. Typical research projects might include the development of decontamination methods, studies of hot core behaviour and removal and experimental work on damaged fuel pellets. "This is an important opportunity to add to the nation's nuclear expertise", Mr Dieckamp said.



"Remember to keep the computer informed every time you wring out your mop!"

AAAS executive foresees greater state direction over science: "I suspect strongly that the 1980s will see the main directions of science and technology decided by government" Mr William Carey, Executive Officer of the American Association for the Advancement of Science, said in Washington last week. Addressing the annual conference of the Society for Social Study of Science, Mr Carey said that the US was already far down that road, since the federal government paid more than half of the national bill for research and development, and influenced the rest through regulatory, tax and fiscal policy. "The illusion of partnership between government and academic science remains a lovely but abstract image, but it is not (if it ever was) an equal partnership". Domestic and international problems meant that this relationship would be "tortured progressively in the coming years". There was likely to be a rapidly growing social preference for centralised government, at the expense of pluralism and checks and balances. And one of the great tests would be that of holding on to such negotiating authority as science still possessed in its bargain with society.

Birmingham University not guilty in smallpox case: Birmingham University was found not guilty last week of health and safety violations arising out of the smallpox outbreak last year that killed Mrs Janet Parker, a university photographer. Parker was working on the floor of the pathogen laboratory of Professor Henry Bedson and the prosecution alleged that Parker caught the disease from airborne particles carried through a faulty ventilation system. Witnesses for the university rejected the argument saying that they were unable to assign a cause to Parker's death other than "personal contact". The UK Health and Safety Executive who brought the suit called the result "disappointing". The HSE is considering an appeal.

UK medical researchers lobby parliament: British politicians who met with representatives from the Association of Researchers in Medical Science on 8 November said that while they sympathised with the problems of short term contract workers they "were not sure that it was a matter for government". One hundred representatives of research workers in the UK marched to the House of Commons after presenting a petition with 1300

signatures to Mr Mark Carlisle, the Minister for Education and Science. A succession of MPs were "amazed" that they had "never heard of this before" but they wanted explanations about the particular situation of researchers compared to other groups in difficulty, why the trade unions weren't involved and they told the representatives that "researchers need to be very much clearer about they want to do". One MP offered to meet with ARMS and the Medical Research Council but when questioned about the efficacy of such a move said that he "wouldn't want to get into a confrontation with the MRC". As a result of the meeting ARMS will prepare a detailed brief of its proposals for a career structure for non-medically qualified researchers by Christmas.

Two thousand protest reorganisation of French biomedical research:

In an open letter to the Prime Minister, 2000 French biomedical researchers have opposed "with profound emotion" the new governmental directives on scientific employment — particularly in INSERM, the national institute of health and medical research. Three hundred researchers demonstrated outside INSERM on 6 November for the meeting of its Administrative Council. According to *Le Monde* the signatories include 195 university professors and hospital doctors, 205 directors of research (including 89 INSERM unit directors), 81 heads of hospital services, 13 presidents of teaching and research establishments, 4 professors of the Collège de France and "one Nobel laureate". The principle points of contention in the new directives are the age limit on recruitment (27 years for researchers, 30 for doctors), a "forced mobility" plan which would give the government the power to assign researchers to any laboratory and new restrictions on promotion when researchers are needed on "priority projects".

Space Shuttle faces further delays — and legal probe: Nothing seems to be going right for the Space Shuttle, which the US National Aeronautics and Space Administration plans to have flying by the middle of next year. Last week another engine was severely damaged during testing at NASA's National Space Technology Laboratories in Bay St Louis, Missouri. No official statement of the impact on the Shuttle programme has been made, but it could delay the first launch, already a year behind schedule, by a further two to three months.

On top of this, the US Department of Justice announced last week that it was investigating charges that Rockwell International, the main contractor for the Space Shuttle, had hidden cost overruns on development aspects of the programme in later production budgets — and that this may have contributed to the delay in the cost-overruns now estimated at 20% above the initial estimate of \$5.15 billion.

US Congress approves \$20,000 million programme to develop synthetic fuels:

President Carter won a major victory for his energy proposals last week when the US Senate approved administration-backed legislation to spend \$20,000 million on the development of synthetic fuels over the next five years. The Senate also approved expenditure of a further \$14,000 million for conservation measures and the use of solar energy and gasohol — including a commitment to speed the development of fuels from agricultural and forest products, a move enthusiastically supported by the US farming community. The Bill approved by the Senate, which has been sent to the White House for signature following the passage of a similar measure in the House of Representatives last Friday, sets a target goal of 1.5 million barrels of oil equivalent of synthetic fuels a day by 1995. President Carter had initially proposed spending \$88,000 million on synthetic fuel development over the five years, but subsequently gave his support to the reduced programme. Included in the programme is an Energy Security Corporation which could build three government owned synthetic fuel plants.

Views of China are as diverse as its visitors. Here Tilman Spengler, who is responsible for scientific exchanges between China and West Germany's Max Planck Institutes, argues that the positive aspects of the Cultural Revolution have been forgotten, while Ken Hsu, (opposite), an expatriate geologist just back from China, lambasts that period and welcomes its passing. In a series beginning later this year, our China correspondent T B Tang — who has also recently visited China — will describe how science remains an intensely politicised activity, despite the impression in the West that the 'Four Modernisations' are loosening overtly political constraints.

China: the legacy of the Cultural Revolution



Involving workers in science during the Cultural Revolution

"THE Gang of Four", a Chinese solid state physicist told me happily in early 1977, "have always insisted that Marxism provides the foundations for solid state physics. That has changed. Today we can again point out that Maxwell equations are of some importance too".

The scientist had the laugh on his side. The other side, the 'Gang', eponym in today's China for every irresponsible ideological nincompoop, had lost their final battle. With the purge of 'The Four' in October 1976 the Cultural Revolution had for most practical purposes come to an end. As time went by, more and more tales of horror about persecutions and harassments of scientists, and accounts of vandalism in laboratories and libraries were revealed in the Chinese press (and to visiting colleagues from abroad). The message — at least as far as science was concerned — left little room for doubt: the Cultural Revolution hadn't just led to a mess, it had ended up in a (sometimes bloody) disaster.

The stories are indeed frightening even if some of them do contain exaggerations — and they should make some Western observers (including an uncomfortably high percentage of natural scientists) think at least twice how they could have eagerly adopted and retained the image of a happy Chinese scientist, cheerfully abandoning his or her research in, say, astrophysics, and discarding all social privileges to become part of the labouring many.

There may have been some exceptions, like a biochemist I met last year in Shanghai who preferred working on pesticides in the

countryside to solitary confinement in his unit (he is by training an expert in neuro-physiology). And there are others who admit that "it did us good" to experience the hardship of having to plant rice sprouts ten hours a day in a paddy field, the common lot of many of those who provide the funds for whatever research is being conducted in the country's laboratories. On the whole, however, the leaders of the Cultural Revolution were apparently singularly unsuccessful in their dealings with China's scientific community. The revolutionaries had a clear idea of what they did *not* want: elitism among intellectuals, international orientations, discipline rather than project oriented research, a system of social values that would perpetuate the division between manual and mental labour, material rewards, and so on. And they hoped to overcome all the traditional obstacles to a "planned science" geared to the country's socio-economic developments by creating the model of a selfless scientist who, by the very nature of his motivation 'for the cause', could do without the common academic incentives (reputation and to lesser degree remuneration).

As we have seen, sticks rather than carrots were used — sticks that Red Guards and a new generation of cadres had little hesitation in applying to individuals and institutions alike. And the Academy of Sciences, hitherto the most powerful and prestigious research organization of the People's Republic made no exception to the rule.

But was that all there was to it? Is the

Chinese press justified in blacking out the decade between 1966 and 1976 as the uncontrollable reign of a few malevolent poltergeists, beset by chauvinism (or at least parochialism) and bent on keeping China in a state of unenlightened autocracy?

A number of facts contradict this new projection. Take, for example, the issue of internationalism. Most of the contracts between the Chinese Academy of Sciences and foreign research organisations, leading to exchange programmes of varying intensity, were begun long before October 1976. Another example is the issue of fundamental research, claimed to be one of the main targets of the science policy of the 'Gang'. Yet high-energy physics has been a priority in Chinese science politics ever since 1972 — and it takes a rather sophisticated understanding of the 'needs of the masses' to include high-energy physics. There were also a number of institutes within the Academy that worked very well, including the Institute of Botany, located in Kunming and set up to investigate the tropical plants of Yunnan province. Even by western standards, this institute is (and was) well equipped, combining extensive empirical researches with highly competent analyses in pharmacology. Others were the Institutes for Vertebrate and Invertebrate Palaeontology in Beijing (Peking) and Nanking, or, to move to the social sciences, the Academy's (now the Academy for Social Science's) Institute for Archaeology, again in Beijing. Of course, these examples cannot be multiplied at will,

but they might make us pause for a moment before we join the chorus of lamentation.

And one should not pass lightly over the various mass campaigns to integrate research, education, health and safety, and production. Again a few examples: in mathematics, it was the "Movement for Operational Research" (yunchouxue yundong), and in oncology the emphasis on epidemiology. One could further add mass experiments and observations in plant pathology and earthquake research.

Let me briefly elaborate the last-mentioned example. Even before the Cultural Revolution, earthquake research had included a variety of specialists, such as mathematicians, geophysicists, geologists, seismologists, architects and historians. Their projects ranged from the adaption of seismographic scales to the peculiarities of the structural engineering aspects of Chinese buildings, to the compilation of a chronology of earthquakes in the history of China which would then be used to divide China into different earthquake regions, as well as planning apartment houses and public buildings which would permit maximum resistance to earthquakes. During the Cultural Revolution this project was extended to include research teams of botanists, zoologists, and meteorologists. They investigated striking changes in fauna or flora which had traditionally preceded

earthquakes, and changes in the ozone content of the air, which likewise serves as an empirical indicator for the threat of an earthquake.

Taken by itself, the addition of the last named group would hardly have constituted any startling departure from the previous forms of organisation. The decisive difference resulted from the widening of the area of inquiry to a level where literally "the masses" could participate. This type of horizontally and vertically integrated research was aimed not only at coordinating many individual talents to deal with a given problem (a social problem translated into a scientific problem), but was also intended — and this was the special message during the Cultural Revolution — to emphatically stress the importance of practical experience, its identification with science and its value for new orientations of action. Thus it encouraged a significant number of peasants to set up and maintain, in their own villages or production brigades, observation stations for early warning signs or earthquakes, on which they provided data. This brought about quite a considerable change of consciousness. Ordinary day-to-day routines acquired the dignity of scientific observations, and as a result reduced much of the traditional mistrust in, and resistance to, 'science' as something alien to their concerns. Equally

important, the adherence to mythological or cosmological explanations which had enjoyed widespread popularity in the Chinese countryside, declined decisively. Natural phenomena had become interpretable in a new context. And this had a third result: the increased survival chances of a people who had learned to replace fatalism by practical planning.

The case of earthquake research probably serves best to illustrate the promises and the limitations of the Cultural Revolution's policy. The promises I have demonstrated: a research that would overcome disciplinary boundaries, answer to a huge social demand, integrate the participants and demanded no great financial investments.

But the limitations are equally clear. Taken as a paradigm for the only legitimate type of research, it would yield results only in those problem areas where everyday data play an important part in the solution, thereby leaving most of the 'mature' disciplines at the wayside. And 'maturity' is under no political conditions an argument for disposal, as the Chinese have had reason to find out.

Tilman Spengler

Tilman Spengler is a sinologist working at the Max Planck Institut für Erforschung der Lebensbedingungen der Wissenschaftlich — Technischen Welt, Starnberg, West Germany.

THE vast arms of China's State Bureau of Geology (SBG) embrace 29 provincial bureaus, more than 20 research institutes, numerous exploration teams, and several institutions of higher education in earth sciences in addition to its headquarters at Peking. Earlier this year, I spent five and a half months as a 'guest expert' of the SBG, and I visited several of these branches in my capacity as a non-paid consultant to help 'modernise' geological activities in China. Usually oral presentations by Chinese colleagues, on which I was expected to give my opinions freely, were followed by outdoor discussions in the field. I would also sit with senior scientific staff and administrators to work out plans to orient future activities. Other duties involved lecturing, including a four-week course on plate tectonics and sedimentology to more than 400 geologists from 91 organisations and hours of subsequent informal discussions.

It has been estimated that China has more than 20,000 geologists. I could not have met more than 10% and talked to more than 1% of the total, and my sampling was not random, so my evaluations should be biased on the optimistic side. However, the reports I heard were not show-pieces, as were presented to us in 1977 when I was a

Ken Hsü — pictured above right — is a Chinese Scientist working at the Eidgenössische Technische Hochschule, Zurich. He visited China last August.



member of the visiting delegation of the International Union of Geological Sciences. I am, therefore, inclined to believe that I did manage a glimpse beneath the superficial during this particular trip.

If I had to use one word to characterise the level of the geological profession in China, the word would be uneven. Our Chinese colleagues are rather up-to-date in offshore exploration geophysics, exploiting much modern equipment, and it is not surprising that their efforts here have been repeatedly successful. On the other hand, sedimentologists in the same office

were still using the methodology of the 1930s. *Erratic* is an apt word to describe the equipping of laboratories. They are commonly well endowed with instruments to analyse material properties of samples, but inadequately equipped to gather data that promote understanding of principles, or of geological processes. In an organisation where Quaternary studies form the essential basis for stratigraphical interpretation of potentially oil-bearing strata, its laboratory is not equipped for paleomagnetic studies and does not have a scanning electron microscope for

nannofossil investigations. Its pollen-laboratory is also inadequate, yet a neutron-activation apparatus has been acquired for microchemical analysis.

There is also a woeful lack of coordination among institutes. Each organisation would like to have its own 'small and complete' laboratories. Administrators were often faced with requests from several institutions for the same expensive equipment, which none of them could utilise fully. The idea of joint ownership and joint research projects among various SBG units was not explored, not to mention cooperation between SBG geologists and those of other systems. The situation became painfully obvious at Tsingtao, where I had contacts with marine geologists and oceanographers from four different systems. Funded separately, each may be able to purchase equipment of the 1950s and 1960s vintage, yet none considered seriously the possibility of combining resources to set up a common up-to-date facility for a national programme on oceanography.

Less visible, but more serious than the problem of modernising equipment is the need for modern methodology. For example, the basic tools of a field geologist — hammer and compass — have remained unchanged for the last century, and it was thus difficult to convey the idea that the methodology for field geology had made advances until we went to the field together. There, I could point out the irrelevance of worrying if a boundary between formations X and Y should be drawn at the base of a bed A, or a few metres higher. Nevertheless, it was not an easy task to persuade our young colleagues to give up their naïve reliance on specific indices, for example that glauconite proves marine deposition even if only a trace is found after a week of searching.

Some Chinese regard this tendency to equate scientific research with routine data-collection as a relic of the Soviet influence. Following the Soviet lead, the People's Republic has adopted the policy of separating teaching (universities), research (Academia Sinica), and practice (Ministry of Geology). There is also a tendency towards premature specialisation: a student has to choose a major in one of the 10 or 15 subdepartments (such as hydrogeology or geophysical prospecting) upon entering the university.

The Soviet ideology had also a depressing effect on senior scientists who had studied in Europe or North America. Many in fact retained their positions after the liberation, but they did not seem to have received the complete trust of Chairman Mao, who repeatedly referred to the need to re-educate the bourgeois intellectuals. Some were down-graded in the aftermath of the 'Let a hundred flowers blossom' campaign of 1957; others were weeded out during the anti-rightist campaign of the late 1950s and early 1960s. Virtually none of the senior scientists

survived the Great Proletarian Cultural Revolution (GPCR) of 1966-1976 unscathed, but the government was so successful in their cover-up that I learned very little of the actual happenings of the GPCR when I visited China in 1976 and 1977. The present governmental policy is not to hide the truth, however, and many of the former victims can now come out with all their woes. Physical punishment, mental torture, forced labour, and imprisonment were the lot of all intellectuals who were occupying high academic positions at the start of the GPCR; I encountered no exception among my relatives, friends, and acquaintances. Many died of suicide, or of poor health.

I consider that the persecution of the intellectuals by the Gang of Four during the GPCR was comparable to the persecution of the Jews by the Nazis during the 1930s: Red Guards or rebel cliques went about freely to search homes, to beat, insult, or imprison people. They set up kangaroo courts which could pass death sentences, without any regard to due processes of the law. There were, for example, ten such 'courts' in the University of Nanking alone! Those, such as my sister, who sat in isolation cells could see no one, talk to no one, and read nothing but the four volumes of Mao's Selected Writings. One of my former schoolmates at the University of California, who returned to serve China in 1954, was kept in a damp cellar for six and a half years, because the rebel clique believed that only a CIA spy could leave the comfort of the US to return to China: patriotism had no meaning for those cynical opportunists. (This friend is now nursing a weak heart complicated by rheumatism.)

Also persecuted during the GPCR were the middle- and high-level cadres. According to the slogans then, these cadres were the 'revisionistic capitalist-roader' who enabled the 'reactionary academic authorities' to betray the Revolution. Very clearly, Lin-Piao, the Gang of Four, and their followers got rid of most of their opposition. Not only were all the province chiefs changed, but all administrators down to the level of administrative assistant of a research institute or university department were at one time or another imprisoned or sent to labour camp. Productive work was suspended completely for the three years to 1969.

When operations were resumed, the men in charge were the so-called 'Worker's Representative', or 'Soldier's Representative', who had no knowledge of the work to be done. The dictatorial Soldier's Representative of the SBG, for example, declared that the practice of doing field-work during the summer and laboratory-work during the winter was a bourgeois soft-practice. Therefore, he forbade the return of field geologists to the laboratory even when the ground was snow covered. To carry out Lin-Piao's order for decentralisation, research institutes and universities were sent to the country in the

provinces, where there were not enough buildings or facilities to permit normal operations. The famous Geological Institute of the University of Peking, for example, was moved to a village in Hupeh, where there was not enough space to open the packing cases, not to mention the inadequate classrooms and housing for staff and students. Consequently the institute had to remain inactive until it was sent to Wuhan after the downfall of the Gang of Four.

The lot of the young scientists was not much better. They were obliged to join one or another of the rebel cliques, which were often engaged in armed battles. At the height of GPCR, young and old professionals were forced to perform the 'Loyalty Dance' and to sing songs like schoolchildren in praise of the Great Chairman. For several years, they could not read anything other than the four volumes of the Selected Writings. They could gradually return to their work in the early 1970s, under the dictatorship of the soldier's representatives who thought of geological exploration as battles to be fought, and issued orders such as: "Take that hill yonder!" The Chinese scientists were thus completely isolated from the international community for ten years.

I may have painted a horror picture of the past, and made an unflattering diagnosis of the present. However, I am optimistic of the future. The present regime has indicated the will to search for solutions to problems, and in this second campaign of "a hundred flowers", there is a liberation from fear. Free and creative thinking is now tolerated, even encouraged, rather than condemned as 'capitalistic decadence'.

During my discussions with administrators, I was encouraged by the fact that funds are available, and steps and being taken to modernise two sedimentological laboratories of the SBG. New centres with the most modern equipment will be established for paleomagnetic and for stable-isotope studies. With the concentration of financial investment and manpower of a socialist system, projects which are not practicable in capitalist countries could be carried out in China. For example, deep drilling of lakes is to take place in order to obtain continuous record of climatic changes during the Quaternary; and continuous coring of a four-thousand metre deep offshore well is scheduled to provide a Neogene reference section — something no capitalistic oil company could be persuaded to do.

My greatest consolation, however, is an awareness of the intellectual quality of the geologists and the administrators to whom I talked. After having had to fight tradition-bound arrogance for the last dozen years here in Switzerland, it is refreshing to find people willing to listen to logical arguments for innovations.

Ken Hsu

"We have a greater degree of social responsibility than UK scientists"

Joe Schwartz talks to three Chinese scientists visiting the UK about their experiences of research and politics in Britain and China.

MS KAO TSE, aged 46, a chemist from Shanghai, Mr Hsu Tan-lin, aged 38, an electrical engineer from Peking and Mr Tsang Shao-hsien, aged 40, a geophysicist also from Peking are three of a group of 12 scholars in the age group 35-50 that are now visiting the UK. Sipping green tea poured out of a large thermos flask, we discussed differences between Chinese and UK science in the plain quarters of the Chinese cultural mission in West London, far from the smart foreign embassies of South Kensington.

The training of all three has been on the European model. Ms Kao, the daughter of a university professor, attended Fudan University, Shanghai's largest with 4500 students. She specialised in chemistry, the curriculum consisting of inorganic chemistry, physical chemistry and organic chemistry with mathematics up to calculus, two years of physics and a "small amount" of quantum mechanics — a standard Western course of study in 1953 the year she graduated. After teaching in Fudan University for a year she attended Peking University for two years post graduate training in inorganic chemistry. She rejoined the staff at Fudan University with an MSc in 1956 as an assistant instructor and was promoted to lecturer in 1959, a position she has held since.

Kao has worked on a variety of research projects but since 1973 her main work has been on the use of rare earth zeolites as catalysts and as molecular sieves. Zeolites are naturally occurring alumina silicates found particularly on the ocean floor that are used as catalysts in cracking crude oil an area of international concern. Zeolites are also useful as molecular sieves in petroleum by-product refining. Kao is spending two years at Imperial College in London in the laboratory of Dr Lovat Rees, a UK specialist in zeolite research. "I came from a large research group of 25 people in Fudan University. We have similar facilities but on a smaller and less well developed scale. At present China must import its larger instruments so we are behind particularly in the use of Mossbauer spectroscopy and in the use of computers.

"I didn't feel at all strange working at Imperial College and I was able to start work right from the first day. We still have a lot to learn from scientists in the UK — more advanced experimental methods and a broader knowledge of related fields. But there isn't that big a gap."

Hsu, the son of a Shanghai metal worker is working on aircraft navigation technology at Imperial College. His home institution, the Peking Aeronautical Institute sent him to the UK "to get current

with the most advanced technology in the field." His brief is digital signal processing using large integrated circuits and computer processing. Digital signals are considered to be far more reliable and easier to work with than analogue signals. Hsu thinks that China can catch up in this field within five years.

He is conversant with the debates about indigenous versus imported technological development. "The government's policy is still one of self-reliance because you can't have political independence without economic independence. We want to introduce microprocessors without becoming dependent on foreign imports. There are already many people in China working on microchip technology and we now can produce some of the chips. We are using the technology that we buy as learning equipment with the ultimate aim of producing it ourselves."

Tsang Shao-hsien's is an earthquake specialist. His work as lecturer in geophysics at Peking University has concentrated on earthquake prediction. It is an area with extensive and unique mass participation. According to Tsang there are now 10,000 professionals and "many more" amateurs engaged in this research with every province having its seismology bureau and brigade. In this field Tsang feels that the positive aspects of the Cultural Revolution were felt more strongly than in more laboratory based work. "Earthquake prediction is in a primitive state all over the globe. In China, the professionals understood that they needed help from the people. The Cultural Revolution posed the question of theoretical work versus practical work and before 1975 'mass work' instead of professional work was emphasised. In 1973 our efforts were to build more instruments and organise more people. China has good social organisation

and earthquake research lends itself to this since the science and the socio-political work can be organised together."

Tsang thinks, however, that theoretical work has been neglected. He has been in the UK for two years familiarising himself with UK theoretical work in the field.

On the wider questions of science policy, education and economic development Kao, Hsu, and Tsang were forthright but at the same time circumspect in the expression of their views. All three believe in quality education. Kao was particularly impressed by recent UK Conservative policy in this area. "I think the Thatcher policy of emphasising aid to the independent schools is important. Some schools should be better than others. In China we have a policy of selecting 'key schools' to make an example for the others."

None of the three saw any danger in the possible domination of education by a small group of intellectuals and party officials. Present policy is that graduates from middle school at age 16 can apply for three years further schooling through competitive examinations set by each city. This is to be compared to policies adopted during the Cultural Revolution when university places went to students elected from their place of work. The example of the Shanghai Machine Tools Factory was important enough for Mao to refer to it in a policy statement on science education in 1968: "It is still necessary to have universities; I refer here mainly to colleges of sciences and engineering. However it is essential to shorten the length of schooling and to make it more political as the Shanghai Machine Tools Plant has done in training technicians from among the workers. Students should be selected from workers and peasants with practical experience and they should return to production after a few years study".

Kao, Hsu and Tsang were familiar with this policy but dismissed it as being "unrealistic." "We would like to enroll all middle school graduates but we must select" says Tsang. Hsu feels that the arguments put forward during the Cultural Revolution were "worthless" and that scientists must "concentrate their efforts on doing scientific work" instead of "being occupied with politics."

When asked whether they saw any differences in the way the UK organised its scientific work compared to China, Kao answered for the group. "In China we tend to work more collectively. It is unheard of for an individual to work alone whereas this is quite common in the UK scientists. Here in the UK, scientists do their work because it interests them. But in China we have adopted the saying "serve the people" as our guiding policy. We believe that the work should be useful first and interesting second. Otherwise it is not good for the human being." □



Workers at the Shanghai Machine Tools Plant: a model for training technicians

One culture bridging two

Samuel Ting, discoverer of the J particle, is a Chinese scientist living and working in the West. Here he talks to **Christine King**

SAMUEL Ting is an American citizen by "an accident of birth". His parents, both professors, were on a visit to Ann Arbor, Michigan, from China in 1936 when he was born. Ting says "my parents had hoped that I would be born in China, but I was born prematurely. Two months after my birth we returned to China." During those early years, China was a country torn by war, so formal education was impossible until he was twelve. Ting remembers now that this lack of continuity in education was compensated by meeting the many scholars who frequently visited their home. Ting's present reputation for hard work and being a highly demanding task-master has its origins perhaps in these early years.

"Since both my parents were working, I was brought up by my maternal grandmother, who was a very courageous and determined person. My grandfather had lost his life in the first Chinese Revolution. After that my grandmother returned to school, became a teacher and brought up my mother alone. When I was very young, I often heard stories from them recalling the difficult lives they had, and the great efforts they made to provide my mother with a good education. Both of them left an indelible impression on me."

Ting attended schools in China and later Taiwan, and decided to return to the US to continue his education at the University of Michigan. "At the time, I knew very little English and had no idea of the cost of living in the US. In China, I had read that many American students go through college on their own resources. I informed my parents that I would do likewise. I arrived at Detroit Airport on 6 September 1956 with \$100, which at the time seemed more than adequate. I was somewhat frightened, did not know anyone and communication was difficult". These were hard years for Samuel Ting, competing constantly for scholarships.

In 1963, with a doctorate and as a Ford Fellow, he went to CERN (the European organisation for nuclear research in Geneva) and came under the guidance of Giuseppe Cocconi, working with the Proton Synchrotron.

After this initiation into European high energy physics, Ting returned to lecture at Columbia University. Here he worked with leading physicists including Steinberger, Schwartz, C.S. Wu, and T.D. Lee. It was during these years at Columbia that his — then unfashionable — interest in electron-positron pair production began.

At the Cambridge Electron Accelerator an experiment on e^+e^- pair production in photon collisions with nuclear targets appeared to show a violation of quantum electrodynamics. (QED is the relativistic quantum theory of electromagnetism; it

remains the most accurately tested of all physical theories.) Ting studied the apparent violation with increasing interest and eventually asked Willi Jentschke of the DESY (Deutsches Elektronen Synchrotron) laboratory in Hamburg about doing a pair production experiment at Hamburg. By this time Ting was fully committed to investigating the decay of photon-like particles, and searching for new particles which decayed to electron or muon pairs. In 1971 he returned with his group to the US and began experiments at the Brookhaven National Laboratory. It was here, in late 1974, that Ting and his group found evidence of a new, totally unpredicted and long-lived heavy particle, the J or psi-particle, which was subsequently shown to contain charmed quarks. For this work, Ting shared the Nobel Prize for physics in 1976 with Burton Richter.

With Ting's growing reputation as a leading physicist have come new problems. In the present political climate of growing intercourse between the US and China, Ting's participation is inevitable. His group at DESY was amongst the first to receive physicists from the Institute of High Energy Physics in Peking to work in the West. The Chinese Press consistently refer to visiting scientists like Ting, and fellow Nobelists Yang and Lee, as Chinese-American or just American. Ting however, is a totally apolitical figure. He categorically repeats "I am not interested in politics and never have been; I have never voted (in the US)."

On his trip to China a few weeks ago, Ting was feted by the Chinese Academy of Sciences (CAS), and as on previous visits, talked privately with Chinese leaders Chairman Hua and Vice-premier Fang Yi, who is also president of the CAS. They talked of China's modernisation programme but, Ting says, they have never discussed political issues "probably because they already know my views".

Ting believes that since he goes to China to communicate with Chinese physicists, there is no time to sightsee. Each of his visits has been widely reported in the Chinese press, and every time his lectures have been attended by thousands. At first he had difficulty lecturing in Chinese (he has spent more of his life on the West than in China): "my audience of several thousand was hardly able to contain their mirth, and corrected me several times on scientific terminology. But I persisted and now it's no longer a problem."

To the outside world, China's decision to pursue high energy physics as part of its modernisation programme, is, to say the least, puzzling. "As I see it" said Ting, "although particle physics appears very complex, its fundamental principles are



Samuel Ting: not interested in politics

nevertheless extremely simple . . . Major discoveries are made by only a few individuals and China has plenty of these. It is an expensive pursuit, but it's also the most fundamental — and it's cheap by comparison to what's spent on defence, say." He believes that the Chinese will maintain their independence in high energy physics. But China's future scientific progress is not a subject which he, or anyone else outside China would feel qualified to predict.

Ting belongs to a new generation of jet-setting academics, spending much of his time at DESY, where he is pursuing the search for the intermediate vector boson at PETRA, currently the world's most sophisticated electron-positron colliding beam device. To keep abreast of his commitments at MIT where he is professor of physics, he flies between Boston and Hamburg on average once a fortnight.

Despite his reputation for working his team hard, no-one works harder than Ting himself. How does life in Hamburg differ from his life in the US? "My life in Hamburg is work, work and more work. As for my team, I try to choose only those who also like to work hard." Interviewing Ting can be a formidable experience if one expects any human foibles — in his own words, he does not smoke, drink or gamble, nor does he read fiction. His family now lives in the US, although the children spent many years in Germany. When he arrives home, do his children complain that they don't see enough of him? "Yes, all the time." Like many Chinese fathers, Ting is determined that his children should put their studies first. When I remarked that in talking of his children, he had repeatedly said only how he expected them to work hard and do well at school, Ting gave a rare laugh. "It's true — around the block where I live, I'm known as the 'bad guy', because I'm so strict with them."

When Ting is not pursuing theoretical particle physics, his predilection is very much towards history. He regards Faraday as a notable historical figure, admiring not only his great breadth of vision but also the difficult and determined path by which he achieved his mastery of science. To be seen as another Faraday — no doubt that would be Samuel Ting's dream. □

news and views

The solar wind connection

from Alan Johnstone

THE solar wind is deflected around the Earth by the Earth's magnetic field. The electrical currents which flow in the boundary layer between the solar wind and the magnetosphere shield the solar wind from the Earth's magnetic field. In 1961, Dungey suggested, that, if the magnetic field, of solar origin, in the solar wind was opposite in direction to the Earth's field at the boundary (called the magnetopause) then the two magnetic fields would diffuse into each other and some geomagnetic field lines would become connected to solar wind field lines (Fig. 1). The geomagnetic ends of the field would be dragged behind the Earth by the solar wind to form a long magnetospheric tail. In the tail the geomagnetic field lines would become reconnected again, and return to the interior of the magnetosphere. The plasma flow driven by this process transfers energy from the solar wind to the magnetosphere, and if sufficient magnetic connection occurred it could produce magnetic storms and auroras.

It is now known that some geomagnetic field lines connect to the solar wind, that the pattern of plasma flow is similar to that driven by such a process and that geomagnetic activity is greater when the solar wind is more nearly opposite the Earth's field (that is, southward directed). Until recently there has been no evidence on the microscopic scale from spacecraft that connection takes place at the magnetopause.

The fields and plasma flow near a region of magnetic connection are shown in Fig. 2. The shielding electric current J flows out of the paper. In a closed magnetosphere the magnetic field on both sides is parallel to the surface. The signature of magnetic connection is a small component B_n normal to the boundary. Although spacecraft magnetometers are very accurate the local surface plane of the magnetopause cannot

be determined from a single spacecraft, or even a pair like ISEE (International Sun Earth Explorer) 1 and 2, so that a small normal component cannot be reliably identified.

Another indication of magnetic connection is an electric field E tangential to the magnetopause. The electric field is more difficult to measure than the magnetic field in tenuous plasma and the first measurements at the magnetopause were not made until 1977 by ISEE 1, and 2, more than 13 years after the first observations of the magnetic field. Mozer *et al.* (*Geophys. Res. Lett.* 6, 305; 1979) have reported the existence of a tangential electric field of the order of 2 mV m^{-1} during a series of crossings of the magnetopause in the subsolar region. There were large fluctuations, taking the value to zero on some occasions.

Heikkilä (*J. Geophys. Res.* 83, 1071; 1978; unpublished work, 1979) has argued that a crucial test for magnetic connection is to find what happened to the electromagnetic energy dissipated in the magnetopause, where $E \cdot J$ is positive (in the particular planar geometry of Fig. 2, E is parallel to J). In the case reported by Mozer *et al.* (*op. cit.*) the power dissipated locally is estimated to be $70 \pm 20 \text{ W km}^{-2}$. This energy must go into the particle distribution. According to theory, plasma flows from both sides towards the boundary where it is accelerated parallel to the surface by the electric motor force $J \times B_n$. In more picturesque terms, it is catapulted out of the region by the tension in the magnetic field lines. Heikkilä (*op. cit.*) showed that the energy deposited in the particle distribution should be observable whether or not this simple picture is correct. Yet, despite many magnetopause crossings by several spacecraft carrying suitable instruments, no part of the particle distribution had been found to be carrying the excess energy until a crossing described in this issue of *Nature* by Paschmann *et al.* (page 243). They have found a crossing

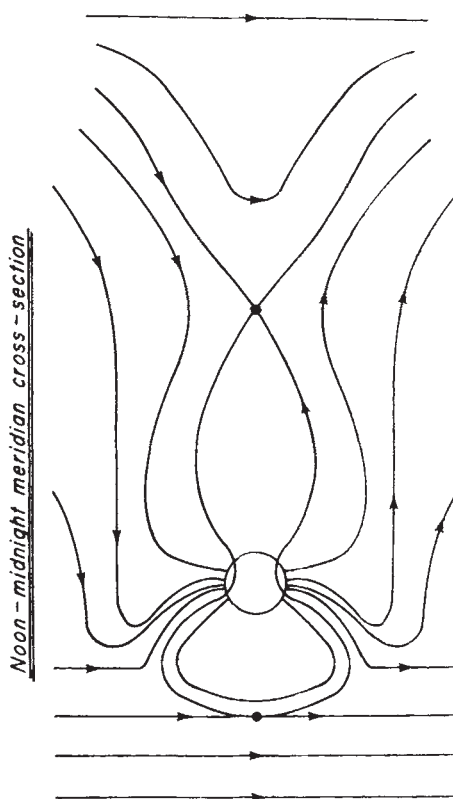


Fig. 1 The magnetic configuration of an open magnetosphere in a southward-pointing, interplanetary magnetic field as proposed by Dungey.

where a high speed flow in the magnetopause is observed by both ISEE spacecraft. The flow velocity of plasma in the layer was 450 km s^{-1} ; much higher than flow velocities in the adjacent magnetosheath and consistent with the simple picture of acceleration by a magnetic catapult. They estimate a power dissipation of $78 \pm 24 \text{ W km}^{-2}$, almost the same as Mozer *et al.* found from electric and magnetic field measurements. Unfortunately, during this crossing, the electric field instrument,

Alan Johnstone is at the Mullard Space Science Laboratory of University College London.

which was on the same spacecraft, was in an unfavourable mode. During the crossing reported by Mozer *et al.* the same particle detectors show no sign of the acceleration (Heikkilä *op. cit.*).

Although the latest result of Paschmann *et al.* provides strong support for magnetic connection it also reinforces the criticisms of Heikkilä (*op. cit.*). If the effect is so easily detected in this crossing why has it never been reported before? If magnetic connection occurs it can hardly be such a rare event as the present observational statistics imply. Is it confined to a small region of the magnetopause or are there special conditions yet to be identified? Other examples, which Paschmann *et al.* say have already been found in the ISEE data, may provide the answers and help us find out what controls the rate of energy input from the solar wind into the magnetosphere. □

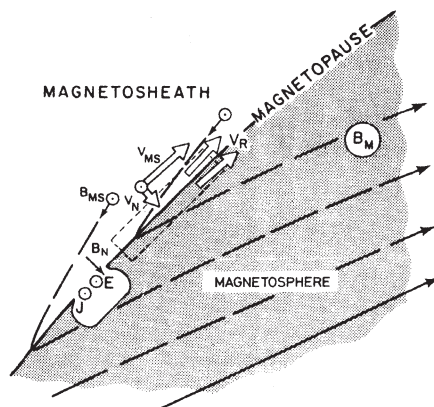


Fig. 2 The configuration of magnetic (B) and electric (E) fields and plasma flow (V) (broad arrows) when magnetic connection occurs at the magnetopause. The enhanced flow caused by acceleration in the layer, V_R , exceeds the normal flow of solar wind plasma past the magnetosphere V_{MS} .

Immunoglobulins and histocompatibility antigens

from Arnold Feinstein

THE family of antibody molecules, or immunoglobulins, for all its magnificent variety and versatility of structure, function and specificity, has evolved by the multiplication of a gene coding for a single ancestral polypeptide sequence, corresponding to a size of about 12,000 molecular weight. As a result of tandem duplication, followed by limited divergence, four or five homologous sequences can be seen repeated along the heavy polypeptide chains of antibody molecules. The κ or λ light chain found in association with each heavy chain is formed from two such homologous regions. In both heavy and light chains hyper-variable stretches conferring antigen-binding specificity are confined to the most N-terminal homology region, the so-called variable region. Crystallographic studies of a few immunoglobulins have revealed that variable and constant homology regions are independently folded similarly, but distinguishably, into individual domains.

What makes it quite certain that the chains of all known antibodies are strings of such similarly folded domains is that all the characteristic conserved features of the folded domain, seen by crystallographers, are easily detected in each of the homologous sequences recurring along all the known immunoglobulin polypeptide chains (see Beale & Feinstein *Q. Rev. Biophys.* 9, 135; 1976). These features include the β strands which form two β sheets, the bends between the strands, and the disulphide bridge lying between the two

sheets and linking them. In this way we have the rare good fortune to be able to come close to the tertiary structure of an immunoglobulin domain from a knowledge of only its primary sequence. Now that the sequences of more proteins are becoming available, the basic immunoglobulin domain structure seems to be cropping up in several unrelated membrane proteins.

It has been evident for some time that another remarkable family of molecules, the classical transplantation antigens (the histocompatibility antigens), are in part coded for by genes which have evolved from the same ancestral immunoglobulin gene although the two loci are completely unlinked. These highly polymorphic antigens are present on most cells, and are extremely important, not only as the critical targets in graft rejection, but also for their involvement in the recognition by cytotoxic T lymphocytes of viral and other cell surface antigens (see *News & Views* 274, 12, 840; 1978). The histocompatibility antigens consist of two non-covalently associated polypeptide chains, the heavier one, which carries the alloantigenic specificities, being a 44,000 molecular weight glycopeptide; the other subunit, β_2 microglobulin, has a molecular weight of 12,000, and its invariant amino acid sequence was found, surprisingly, to be homologous to that of a constant immunoglobulin domain, including the characteristic disulphide-bridged loop. Moreover, all the three-dimensional structural features of this domain referred to earlier can be recognised.

In antibody molecules constant domains regularly associate in pairs,

so that when two disulphide-bridged loops of appropriate size were seen in the heavy chain of the transplantation antigen, it seemed very likely that at least one of these looped regions would be folded like an immunoglobulin domain, and would be associated with β_2 microglobulin. Indeed, the familiar features of a constant immunoglobulin domain could already be seen in a tentative sequence of one of these regions, in a human histocompatibility antigen, which was published last year by Strominger and his colleagues.

In this issue of *Nature* (page 266) Orr *et al.* present the complete sequence of this region, referred to as ac2, and confirm in detail its immunoglobulin-like structure. But what of the other more N-terminal disulphide-looped region? (see Fig. 1 of Orr *et al.* for the relative arrangement of the regions). The sequence of this region too has now been published (*Proc. natn. Acad. Sci. U.S.A.* 76, 4395; 1979) and it is disappointingly apparent that, apart from the sequence around the most N-terminal cysteine, the overall pattern of conserved features typical of an immunoglobulin domain are not clearly seen. Thus, even if this region were derived originally from an ancestral immunoglobulin gene, it has adaptively lost a number of its features. For this reason, ac2 is a more likely candidate for the region with which β_2 microglobulin is associated.

There is however, certain to be a concerted effort to predict a plausible structure for the region N-terminal to ac2, following the realisation that this part of the molecule is important in T cell recognition. This awareness has been highlighted by studies of mutant mice, where dramatic functional changes have been correlated with single amino-acid substitutions in this region (see Kohn *et al. Immunogenetics* 7, 279; 1978). Not only can such mutations lead to graft rejection, but the specificity for T cell recognition of viral antigens seen in association with the original and with the mutant molecule can be quite different.

It appears that each of the different types of polypeptide chain known to contain immunoglobulin or related sequences is controlled by one of five genetic regions, which are not detectably linked in the species studied. It seems that we can now add a sixth. Thy-1 is a glycoprotein which has been isolated by an Oxford group from rat brain, but it is also abundant on rodent thymus cells. Its sequence indicates that Thy-1 resembles a single immunoglobulin domain (see Campbell *et al.* this issue of *Nature*, page 341). Although the function of this molecule is unknown, we should in general resist the expectation that invariant proteins structurally related to immunoglobulins will have specific recognition properties analogous to those of the specialised variable domains of antibodies. We do not know what function the ancestral polypeptide served, but we have every indication that it owes its extensive proliferation, unique in mammals, to its striking adaptability to a variety of functions.

Arnold Feinstein is at the ARC Institute of Animal Physiology, Babraham.

Georg Wittig — virtuoso of chemical synthesis

The 1979 Nobel Prize for Chemistry was awarded jointly to Georg Wittig and Herbert C. Brown. Here Robert Shaw describes Georg Wittig's achievements in synthetic and mechanistic chemistry.

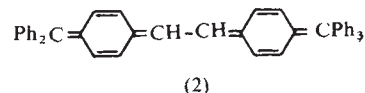
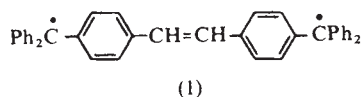
Georg Wittig was born on 16 June, 1897 in Berlin and after his *Abitur* hesitated between chemistry or music; luckily for chemistry he chose the former. His love of music has remained with him however, and in his earlier days he was an accomplished pianist. I should add that he has also been a keen mountaineer with many difficult ascents to his credit.

He commenced his study of chemistry at the University of Tübingen in 1916 but war service almost immediately intervened. He returned (on release from a prisoner of war camp in the UK) to the study of his chosen subject working under Karl von Auwers. In 1926 he obtained his *Habilitation* at the same university. From 1923-32 he was Teaching Assistant (first under von Auwers, then under Meerwein) at the Chemical Institute of the University of Marburg. In 1932, he went to the Technical High School in Braunschweig as *ausserplanmässiger* Professor. He stayed there until 1937, when he became *Extraordinarius* in Freiburg. In 1944 he became *Ordinarius* at the University of Tübingen and left there in 1956 to become Director of the Chemical Institute of the University of Heidelberg. In 1965 he became Professor Emeritus but continued to co-direct the Institute for Organic Chemistry in Heidelberg for a further two years. He has remained active since. This is a bare outline of a brilliant scientific career spanning half a century.

Georg Wittig has received many honours and it is appropriate that his long and distinguished career has been crowned by the award of the Nobel Prize — a recognition which was long overdue.

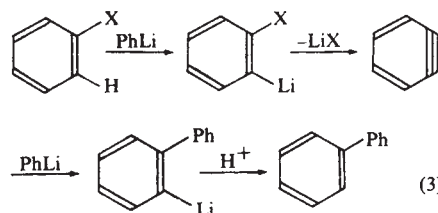
If the impact a scientist makes on his peers is measured by the achievements which carry his name Wittig has scored a hat-trick: the Wittig hydrocarbon, the Wittig rearrangement and the Wittig reaction — an achievement that has been matched by few.

Wittig's scientific work began with studies on γ -pyrones. Investigations on free radicals then followed, in particular, of polyphenylated hydrocarbons. In an attempt to prepare a 'biradical (1), the Wittig hydrocarbon, bis[4-(diphenylmethylene)cyclohexadienylidene]ethane (2) was obtained in 1936.



For these studies Wittig used phenyl-lithium, first used by him in 1931; this reagent recurs again and again in many of his most important discoveries. Wittig called it his *Wünschelrute* (divining rod). His work with organolithium compounds and related organometallic reagents (following earlier work by Schlenk and Bergmann) establishes him, together with Henry Gilman and Karl Ziegler, as one of the fathers of organometallic chemistry of the main group elements.

Phenyl-lithium is a very strong base and nucleophile. Both of these properties are of importance in its reaction with halogenobenzenes, PhX ($\text{X}=\text{F}, \text{Cl}, \text{Br}$) to give diphenyl, PhPh . The observation that fluorobenzene ($\text{X}=\text{F}$) was the most reactive in this series led to the following reaction scheme, including the postulate in 1942 of the then revolutionary idea of a dehydrobenzene (benzyne).

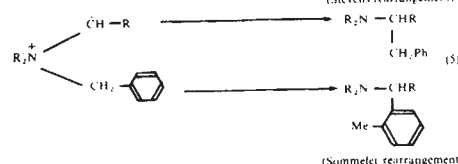


In the 1950s Wittig (and independently John D. Roberts) explored the synthetic and mechanistic aspects of benzyne. His studies were extended to other dehydroaromatics, dehydroheterocyclics and small-membered cycloalkynes in the 1960s. These short-lived intermediates were trapped as characteristic adducts with dienes or bases and this procedure led to novel syntheses. It may be an interesting aside that Wittig and Roberts believe that they are distantly related, as the name Dombrowski, occurred in both their families.

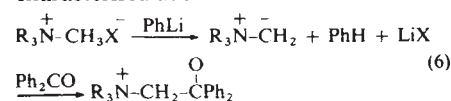
Much of Wittig's work was in the field of organic anionic chemistry. Here he carried out important investigations on metallated ethers (the Wittig rearrangement) in 1942



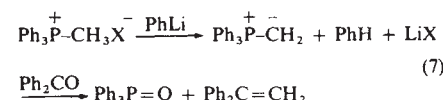
and ammonium ylides 2 years later



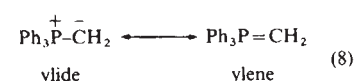
The zwitterionic ylides were obtained from the reaction of phenyl-lithium with quaternary ammonium halides and the unstable products converted with benzophenone to stable and easily characterised adducts.



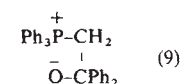
Extension of this work to phosphorus compounds led to the discovery of the phosphonium ylides in 1953, and their reactions with aldehydes or ketones to give the famous olefin synthesis which carries Wittig's name.



This ylide for which also the ylene canonical form is discussed

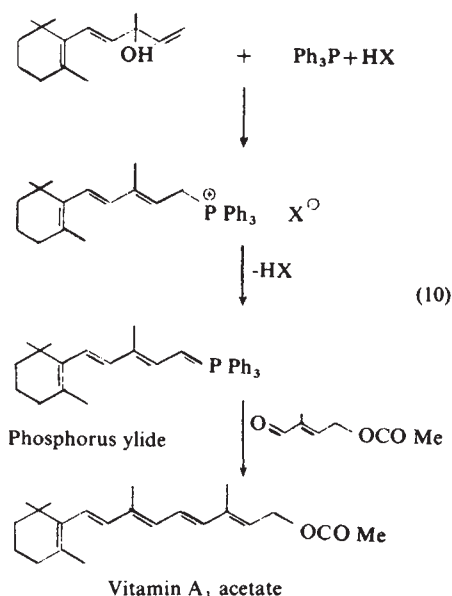


reacts *via* zwitterionic intermediates, usually unstable, but which can be isolated in special cases, to give olefins with a precisely defined location for the double bond.



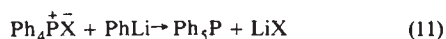
This olefin synthesis proceeds under mild conditions and is thus ideally suited for the synthesis of natural products. Recent work allows considerable control of whether *cis* or *trans* olefins are formed. Amongst the

triumphs of this synthetic procedure are vitamin A₁, β-carotene, citranaxanthin (a food dye), vitamin D₂, progesterone, squalene, the sesquiterpenes α and β-sinensal (the aroma of orange oils), juvenile hormones (with potential in pest control), prostaglandin PGF₂, the Queen bee substance (a pheromone), an optical brightener (PALANIL[®] Brilliant White R) for cotton and polyester fibres, and many more. In particular vitamin A₁ is now produced on a tonne scale by this method. The BASF process for its synthesis is outlined below.



Another contribution by Wittig was the use of organolithium compounds and other strong bases to obtain carbenes in 1959, now widely recognised as important intermediates in many organic reactions. More recently carbenes have been observed in a variety of complex organometallic compounds, and some of these have been characterised by X-ray crystallography.

An α-carbon-hydrogen bond is essential for the formation of phosphorus ylides. Utilising the absence of such a structural feature in phenyl derivatives, Wittig synthesised pentaphenylphosphorane, Ph₅P.



By analogous routes pentaphenyl derivatives of arsenic, antimony and bismuth were obtained, as were tetraphenyl derivatives of sulphur, selenium and tellurium and a triphenyl derivative of iodine.

In addition to the above mentioned neutral organometallic compounds Wittig prepared numerous anionic derivatives, the 'ate'-complexes, and explored their chemistry. Two examples must suffice: sodium tetraphenylborate, NaBPh₄, used

in the estimation of potassium and of some alkaloids, and lithium hexaphenylantimonate, LiSbPh₆.

Science may be impersonal — scientists certainly are not. So I cannot write about Georg Wittig the scientist without mentioning Georg Wittig the man. My first contact with Wittig was when, as a PhD student I wrote to him about a paper of his and Vidal's on the hydrocarbon fluorene and received a most courteous reply. I will recall the kindness with which he later received me and many other young scientists at his Institute in Heidelberg and his and his wife's generous hospitality. His Institute mirrored his personality, courteous and always kind, and was not one where continuous internecine warfare was current: his kindness made him an exceptionally effective teacher to those fortunate enough to have been his pupils. He was an inspiration to all those who came in contact with him.

His wife Waltraut, herself a PhD in chemistry, was his constant and trusted

companion and her unexpected death last year was a severe blow to him. With firmness and kindness she stood by his side and shielded him from most of the more mundane cares of modern life leaving him free to concentrate on his scientific work. It must be a great sadness to him, as well as to those of us who knew her, that she did not live to see his work rewarded with the award of a Nobel Prize.

Georg Wittig's lasting achievements speak for themselves but two aspects deserve particular comment. Most of his work was done without the benefit of modern high technology instruments such as high field NMR spectrometers, four-circle diffractometer and so on. His most important 'instruments' were his vast chemical knowledge, his fine intellect and a superb chemical intuition. And much of his best work was done at an age when for many others the spring of scientific innovation has dried up. Good wine matures with age and Georg Wittig is undoubtedly of the best vintage. □

Protean muscle

from Alan Weeds

MUSCLE is a highly adaptable and responsive tissue. Its physiological and biochemical properties accommodate to changes in functional requirements, altered patterns of nerve stimulus and hormonal variation. Dramatic changes are seen in the pattern of gene expression during embryological development and following denervation or local injury: such systems provide valuable models for unravelling the temporal sequence of these genetic events and elucidating their ontogeny. To what extent does the pattern of nerve impulses control muscle performance? How is this modified by use, hormones and other nutritional factors? What is the mechanism whereby these external influences affect the genetic programme of muscle? Is there a common messenger? These were some of the questions discussed at a recent meeting on Muscle Plasticity*.

Myosin changes during development

Probably the most controversial aspect of the meeting concerned the nature of myosin gene expression in developing muscle, a subject that has been debated in *Nature* recently (see 280, 321; 1979). G. Gauthier (University of Massachusetts, Worcester) described the use of purified antibodies to fast and slow chicken muscle myosins to identify cross-reacting species in developing rat muscles by indirect immunofluorescence. In early post-natal development all fibres of extensor

digitorum longus (EDL, which is fast-twitch in the adult) and soleus (slow-twitch in the adult) stained with both antibody preparations. Segregation of staining into the adult pattern occurred only later, probably coincident with the stage when the muscles cease to be polyneuronally innervated and are stimulated through a single axon. The experiments suggest the presence of both adult forms of myosin within the same fibre or may indicate a different myosin isoenzyme which cross reacts with both sets of specific antibodies. By contrast, N. Rubinstein (University of Pennsylvania, Philadelphia) argued that most of the fibres of both EDL and soleus muscles from 18-day rat embryos stained exclusively with antibodies to fast muscle myosin while only a few stained with antibodies to slow muscle myosin. At birth all the fibres still reacted with anti-fast myosin antibodies, but the level of staining was weaker in those fibres which also reacted with anti-slow myosin antibodies. These experiments, combined with two-dimensional gel electrophoresis of the myosin light chains suggest that only fast muscle myosin is synthesised in both fast and slow muscles at the earliest stages of development. Cultures derived from chick pectoralis and anterior latissimus dorsi muscles *in vitro* (fast and slow respectively) were also shown to synthesise only fast myosin by these criteria.

R. Whalen (Institut Pasteur, Paris) reported evidence for an embryonic form of myosin in developing rat muscles and also in bovine and chicken muscles. This was based on light chain patterns in two-dimensional gel electrophoresis and also on

Robert A. Shaw is Professor of Chemistry at Birkbeck College, University of London.

*The International Symposium on Plasticity of Muscle was held at the University of Konstanz, Germany between 23 and 28 September and organised by Professor D. Pette. The proceedings of the meeting will be published in early 1980 by Walter de Gruyter, Berlin.

gel fingerprints of chymotryptic fragments of the myosin heavy chains. Embryonic myosin is the predominant species in primary tissue culture cells after fusion and is present in newborn rat muscles as a minor species. Thus in soleus muscle of newborn rats, all three forms of myosin occur. J. Hoh (University of Sydney) also provided evidence for an embryonic form of myosin based on gel electrophoresis of the native protein.

While differences in the immunofluorescence results may reflect different techniques and variations in the response of the animals used to raise the antibodies, these different results are at variance, particularly those of Rubinstein, and will require further investigation. Nevertheless, the evidence for an embryonic myosin is persuasive and it is also clear from a number of systems that two or more myosin isoenzymes may coexist within the same fibre. But we do not know whether, if these myosins are synthesised simultaneously, polyneuronal innervation is required or whether this is part of the intrinsic developmental genetic programme. Part of the problem is to establish the precise times at which polyneuronal innervation becomes effective and is subsequently eliminated. Further experiments on the sorting of both myosin and troponin isoforms (discussed by S. Perry, University of Birmingham) are needed to elaborate the complex sequence of myofibrillar gene expression during differentiation and development. E. Kugelberg (Karolinska Institut, Stockholm) showed that there was a steady transformation of fibre motor units from fast to slow in soleus muscles from maturing rats, but whether the intermediate contraction times result from changed discharge frequencies of the motoneurons remains to be proved.

Innervation and regeneration

A related problem, discussed by R. Bischoff (Washington University, St Louis) and A. Kelly (University of Pennsylvania, Philadelphia) concerned muscle regeneration following denervation or local injury. Bischoff suggested two routes. Satellite cells located beneath the basement membrane may proliferate and fuse to form new myotubes or, under conditions where these are killed, dedifferentiation of myofibres occurs, which subsequently form immature myotubes leading to new myofibrils. This demonstrates once again the remarkable versatility of muscle tissue. Kelly argued that the programme of myosin gene expression during regeneration mimicked that occurring during development.

G. Vrbová (University College, London) presented evidence that polyneuronal innervation disappears when the animal starts moving and suggested that retraction

of nerve fibres from the endplate might be due to the action of proteolytic enzymes, stimulated by acetylcholine and calcium ions. Thus maturation of muscle fibres depends not only on stimulation by a single motoneuron but also on muscle use. Other speakers also emphasised the importance of use. D. Goldspink (Queen's University Belfast, Northern Ireland) reported that limb immobilisation produced a marked increase in protein degradation and simultaneous decrease in synthesis, but atrophy was greater in the normally more active soleus muscle than in EDL. Stretching the muscles reduced the rate of atrophy through increased protein synthesis. H. Vandenburg (National Institute of Mental Health, Bethesda) demonstrated increased protein synthesis in cultured muscle cells in response to stretch. He suggested that stretch activates the sodium/potassium ATPase, which might enhance amino acid transport. J. Etlinger (SUNY Downstate Medical Center, Brooklyn) showed effects of calcium ions as well as stretch on protein degradation and biosynthesis in rat muscles. Release of myofilaments during degradation is stimulated in the presence of calcium ions by way of proteolysis. Thus the application of mechanical force influences ion movements in muscle membranes which may be responsible for the observed changes in protein turnover.

The effects of a number of hormones on protein synthesis and degradation were reviewed by A. Goldberg (Harvard Medical School, Boston), emphasising the dynamic nature of muscle as providing 80% of available body protein for metabolism. C. Ianuzzo (York University, Toronto) and A. Montgomery (Newcastle General Hospital, Newcastle upon Tyne) showed the effects of altered thyroid hormone levels on the presence of fast-twitch fibres in rat soleus muscles. Thyroid status clearly influences the pattern of myosin gene expression in addition to its general effects on protein turnover.

Electrical stimulation

The other major area of discussion centred on altered patterns of electrical stimulation and use in adult muscles. Although denervation, spinal transection and cross-reinnervation have provided useful models, these surgical procedures are often complicated and the effects long term. Emphasis at this meeting was on the use of chronic electrical stimulation to investigate the influence of different firing rates on speed of shortening and metabolic enzyme levels. T. Lømo (University of Oslo, Norway) reported chronic stimulation experiments on rat muscles following nerve section, where the only stimulus reaching the muscles was the externally applied one. His most interesting findings were that infrequent 100 Hz pulses applied to slow muscles produced properties characteristic of fast-twitch muscles. But he found that the speed of contraction was regulated

independently from the muscle's endurance. O. Hudlická (University of Birmingham) described effects of chronic stimulation in increasing blood flow and oxidative enzyme levels, while D. Pette (University of Konstanz, Germany) showed dramatic changes in metabolic enzyme activities, especially hexokinase, within 24 hours following stimulation of rabbit fast-twitch muscles at 10 Hz for 12 hours per day. C. Heilmann (University of Konstanz) examined effects on the sarcoplasmic reticulum and found reduced calcium uptake and ATPase activity within 2 days of applying artificial stimulation. In addition there were changes in the composition of phosphorylated proteins in the reticulum membrane. S. Salmons (University of Birmingham) showed an increase in the activity of enzymes involved in polyamine synthesis of nearly two orders of magnitude within 28 hours of applying the external stimulator, which might reflect increased levels of mRNA processing or translation in these muscles. Although this model was discussed extensively, Vrbová suggested that pharmacological agents and hormones might prove more valuable for the future because of the 'insulting' effects of this abnormal form of stimulation on the muscles.

The meeting represented somewhat of a watershed. I left with the feeling that the groundwork has been laid but that there was much more to achieve. Particularly important would be tissue culture systems which were free from the vagaries of whole animal models. A. Shainberg (Bar-Ilan University, Ramat-Gan, Israel) introduced us to novel experiments analysing acetylcholine receptors on myotubes following electrical stimulation. Although the importance of the membrane became increasingly clear as the meeting proceeded, relatively little attention has been paid to it in the past. Whatever the signals may be that control the genetic programme, the membrane acts as their gate. Our mercurial muscles have much yet to reveal. □

Life in a cold climate

from W. Nigel Bonner

THE Antarctic provides a testing environment for many forms of life. Severe climate, characterised by large temperature range and oscillations, with long periods of subzero temperatures, combine with extreme isolation to create a set of conditions where the critical response of an organism to its environment can often be simply expressed in terms of one or two key features. Studies of such responses allow the description of the adaptational strategy of certain polar organisms and are being undertaken by the British Antarctic

Alan Weeds is a member of the scientific staff at the Medical Research Council Laboratory of Molecular Biology, Cambridge.

Survey, (a component body of the Natural Environment Research Council). Some recent work was reported at a conference at the Linnean Society last month*.

The typical Cryptostigmatid mite, *Alaskozetes antarcticus*, which is an important component of the very sparse Antarctic terrestrial invertebrate fauna, is continually exposed to the risk of tissue freezing. Freezing is fatal to all stages of this mite. The risk of freezing is increased if the mites have been feeding, as food remains in the gut provide potential ice-nucleators when supercooled. W. Block and S.R. Young have found that *Alaskozetes* can to a great extent avoid freezing first by ceasing to feed in the autumn, so that the gut is emptied of potential ice-nucleating agents, and second by developing an ability to supercool to -26°C . Supercooling is enhanced by glycerol, which the mite can synthesise as a response to low temperature and low relative humidities, to allow survival to at least -31°C . Juvenile mites possess an even greater ability to tolerate cold than do adults. In this way juveniles and adults avoid tissue freezing in winter in their maritime Antarctic habitat. *Alaskozetes* maintains a metabolic rate some two to three times higher than that of comparable temperate mites over the range 0 to $+10^{\circ}\text{C}$. This elevation of metabolism or cold adaptation combined with its strategy for avoiding freezing allows large populations of mixed instars to survive the Antarctic winter and so use the short summer period for growth and reproduction. Life cycles of such species are necessarily long (up to 2-3 years) in the Antarctic.

Conditions are very different in the Antarctic sea. Here temperatures, although low, are stable in comparison with the terrestrial environment and freezing is a problem for relatively few



Terrestrial mites at Signy Island, maritime antarctic. Adults, nymphal stages and eggs of the detritivore, *Alaskozetes antarcticus* are seen on a rock surface with four individuals of the lighter-coloured predator *Gamasellus racovitza*. (Photograph by I.B. Collinge, BAS.)



Elephant Seal breeding beach. Studies on Elephant Seals at South Georgia by British Antarctic Survey have shown how this species has recovered from the effects of commercial sealing in the past. (Photograph by T.S. McCann.)

organisms (notably fish and some shallow-water molluscs). More important is the great seasonal variation in food availability, caused by changes in light-climate and ice-cover. This results in a brief season of phytoplankton bloom, in which food is abundant, followed by a long period of scarce or erratic supply. Andrew Clarke suggested that Antarctic marine invertebrates, unlike *Alaskozetes*, are characterised by low basal metabolic rates, (regarding basal metabolism as the generation of ATP for protein turnover, for membrane pumps, and (with NADPH) for lipid turnover, plus the cost of circulation). Coupled with low basal metabolic rates are slow growth, reduced annual reproductive effort and by analogy with fish, low rates of protein synthesis. Together, these result in a reduction of the total food requirement, thus facilitating survival in an environment when food is limited for a large part of the year.

The absence of planktonic larval stages of benthic invertebrates is characteristic of the polar seas. In Antarctic prosobranch molluscs, for example, most forms produce a few large yolky eggs from which tiny but perfect snails emerge after a long development. This parallels the principal reproductive strategy of other polar benthic invertebrates and G.B. Picken argued that this style of early development, whether by means of egg capsules, brood protection or viviparity, has strong survival value in localities where low temperature and slow growth mean that planktonic larvae would be unable to exploit the brief abundance of

phytoplankton during the summer bloom. The trend towards reduced fecundity, enhancing the survival of the large, well-developed juveniles in comparison with more numerous but smaller planktotrophic larvae, and the extreme isolation of shallow-water benthic habitats in the Antarctic, may be the principal factors responsible for the high level of species endemism found there.

Sea-birds also exploit the Antarctic environment. Being homeotherms, low temperatures are not primarily a problem for them, provided energy sources are adequate. During the breeding season, however, which is in general timed to take advantage of the high seasonal food availability, food demand is dramatically increased at those sites where the birds concentrate. South Georgia is such a site and J.P. Croxall and P.A. Prince have studied how the various species breeding there divide the food resources between them. In some cases chick-feeding periods are complementary between species pairs, so that direct competition for food between similar feeders is reduced. In others, there may be species differences in the frequency with which chicks are fed, allowing one species to exploit more distant feeding grounds than another. Most commonly, however, differences are found in the detailed composition of diets. The two very similar albatrosses, the Black-browed Albatross and the Grey-headed Albatross, for example, have as principal components of their diet krill and squid respectively. Because squid is less nutritious than krill Grey-headed Albatrosses rear their chicks more slowly and are unable to breed every year. However, in compensation their adult survival is substantially greater than in the Black-browed Albatross. □

*Nine papers reporting recent biological research in the Antarctic by British Antarctic Survey were presented at a symposium at the Linnean Society on 11 October, 1979. The proceedings of the symposium will be published as Vol. 14 (part 1) of the *Biological Journal of the Linnean Society*.

James Clerk-Maxwell—100 years later

Cyril Domb

King's College, University of London, Strand, London WC2, UK

This year is the centenary of the death of James Clerk-Maxwell, whose theory of the electromagnetic field provided the background essential to the development of Einstein's ideas. Einstein, at the commemoration of the centenary of Maxwell's birth in 1931, described the change in conception of reality in physics after Maxwell as "the most fruitful that physics has experienced since the time of Newton".

THE concept of electromagnetic radiation originated with Maxwell, and his field equations paved the way for the special theory of relativity. Newton, Maxwell and Einstein are in a class of their own among physicists, and it is remarkable that Einstein was born in the year that Maxwell died, as in many respects Einstein took over where Maxwell left off. The quantum theory also leaned heavily on Maxwell's ideas. Max Planck introduced his quantum hypothesis to account for the spectrum of 'black-body radiation' (electromagnetic radiation in thermal equilibrium with matter at a given temperature) and to resolve the UV catastrophe of the classical theory for which an infinite amount of energy was present in radiation of short wavelengths. In the subsequent development of the theory of the structure of atoms and molecules and of matter in general, the interaction between matter and radiation has a central role.

Maxwell also made fundamental contributions to other branches of physics, notably thermodynamics, the kinetic theory of gases (the idea of applying probability and statistics to molecules is due to Maxwell), colour vision and colour photography. In the nineteenth century there was no serious division between theoretical and experimental physics. Maxwell engaged in both throughout his life, and his best known appointment is that of first Professor of Experimental Physics in the University of Cambridge.

Contemporary assessment

Maxwell's contemporaries did not think quite so highly of him. When the Cavendish Laboratory was initiated in 1871 Maxwell was only the third of those proposed for the appointment of director. The first was William Thomson (later Lord Kelvin). He did not wish to move from Glasgow and suggested Helmholtz who also refused to move from Berlin where he had just been appointed director of a new laboratory. Only then was Maxwell approached, and the letter of invitation by the Reverend E. W. Blore stated: "It has I believe been ascertained that Sir W. Thomson would not accept the Professorship. I mention this in case you should wish to avoid the possibility of coming into the field against him." When Maxwell did eventually take up the appointment at Cambridge the average attendance at his lectures was between two and three students!

His first academic teaching appointment was at Marischal College in the University of Aberdeen in 1856. In 1860 a merger took place between the two constituents of this University, King's College and Marischal College, and one of the two professors of Natural Philosophy became redundant; David Thomson of King's College was allowed to continue, ostensibly because of superior teaching ability, and Maxwell was declared redundant.

Maxwell applied for a vacancy at the University of Edinburgh but he was turned down in favour of his school friend Peter

Guthrie Tait who was again considered a better teacher. Fortunately King's College London appointed him to the Chair of Natural Philosophy. The five subsequent years were undoubtedly the most fruitful of his career. He was elected to the Royal Society in 1861 aged 29. His two classic papers on the electromagnetic field were published in 1862 and 1864. In 1861 he delivered a lecture at the Royal Institution on the theory of three primary colours, and demonstrated the first colour photograph. The theoretical and experimental work on the viscosity of gases which took place during these years culminated in his Bakerian lecture to the Royal Society in 1866. He supervised the experimental determination of electrical units for the British Association's Committee; this work of measurement and standardisation led to the establishment of the National Physical Laboratory. He also measured the ratio of electromagnetic and electrostatic units of electricity, and confirmed that it was in satisfactory agreement with the velocity of light as predicted by his theory.

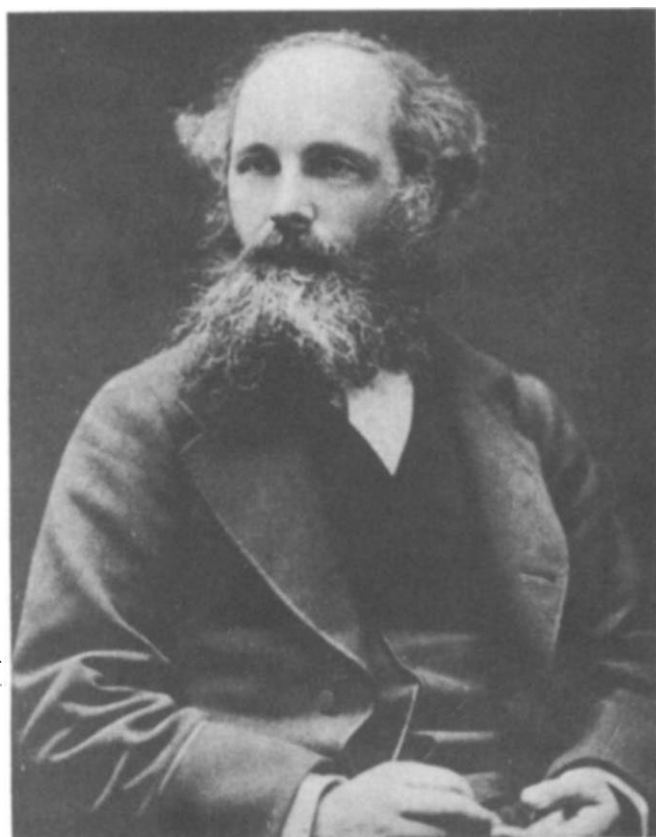
But even the association with King's College was described in negative terms by the College historian. Maxwell resigned his Chair in 1865 and retired to the family estate in Glenlair, Scotland. When the centenary history of the College was published in 1928 it claimed that Maxwell was asked by the Governors to resign because he could not keep order at lectures. There is, however, no hint of such a situation in the biography by Campbell, in the obituary notice in the Royal Society or in any of Maxwell's correspondence. I have recently investigated this claim and found it devoid of any substantial basis.

Maxwell emerged from retirement to take up the appointment at Cambridge in 1871, where he spent his final eight years; he died on 5 November 1879 aged only 48. He published just over 100 papers, received no public honours, and was buried quietly in a small churchyard in the village of Parton, Scotland. By contrast, his friend William Thomson lived on for nearly 30 years more, published 600 papers, received every possible honour and distinction, and was buried with great acclaim in Westminster Abbey.

The true quality of Maxwell's genius became apparent only with the development of twentieth century physics. In addition to the major contributions to science mentioned previously, several ideas which he put forward quite casually have proved extremely fertile, and have led to important advances.

Biographical notes

Fortunately Maxwell's lifelong friend, Lewis Campbell (together with the first Demonstrator in Physics at the Cavendish Laboratory, William Garnett) produced a definitive biography within a few years of his death. Other biographical essays have appeared, the most important of which (by C. W. F. Everitt) provides new biographical material, and reviews



James Clerk-Maxwell in his later years.

Maxwell's scientific achievements from the vantage point of the present day.

James Clerk-Maxwell came from a comfortable middle-class background. The original family name was Clerk, the additional surname Maxwell being added by his father because of the inheritance of the Middlebie estate from ancestors of that name. James, an only child, was born in Edinburgh where his father was a lawyer; his parents had married late in life and his mother was 40 years old at his birth. Shortly afterwards the family moved to Glenlair, the country house on the Middlebie estate.

James showed remarkable curiosity at an early age, and before he was 3 his mother wrote "he has great work with door locks, keys, etc. and 'show me how it doos' is never out of his mouth. He also investigates the hidden course of streams and bell wires. . . . As to the bells, they will not rest; he stands sentry in the kitchen, or he rings, and sends Bessy to see and shout to let him know, and he drags papa all over to show him the holes where the wires go through." He also had a phenomenal memory, and at the age of 8 he knew by heart the longest chapter of the Old Testament (Psalm 119 containing 176 verses).

Alas, his mother died in 1839 from abdominal cancer, the same disease to which he was to succumb at exactly the same age. A dull and uninspired tutor was engaged who claimed that James was slow at learning. Fortunately he was rescued by his aunt Jane Cay and was sent to school at the Edinburgh Academy. Among the other pupils were his biographer Lewis Campbell, and his friend Peter Guthrie Tait.

Maxwell's interests ranged far beyond the school syllabus, and he did not pay particular attention to examination performance. But he published his first scientific paper at the age of 14 describing a generalised series of oval curves which could be traced with pins and thread by analogy with an ellipse. This fascination for geometry and for mechanical models continued through his whole career, and was of great help in his subsequent research.

At the age of 16 he entered Edinburgh University where he read voraciously on all subjects, and published two more scientific papers. He went on to Cambridge in 1850, and here there is evidence that his exceptional powers began to be recognised. H. M. Butler (later Master of Trinity) wrote "When I came up to Trinity Maxwell was just beginning his second year. His position among us was unique. He was the one acknowledged man of genius among the undergraduates." Another friend W. N. Lawson wrote in his diary "Maxwell as usual showed himself acquainted with every subject on which the conversation turned. I never met a man like him. I do believe there is not a single subject on which he cannot talk, and talk well too, displaying always the most curious and out-of-the-way information."

A more discriminating appraisal came from his mathematics teacher, William Hopkins, the famous 'wrangler maker' of the period whose students included G. G. Stokes, William Thomson, Arthur Cayley, P. G. Tait and E. J. Routh. Of Maxwell he is reported to have said that he was the most extraordinary man he had met with in the whole course of experience; that it seemed impossible for him to think wrongly on any physical subject, but that in analysis he was far more deficient (other contemporaries testified to Maxwell's preference for geometrical over analytical methods). This shrewd assessment was borne out later by several important formulae advanced by Maxwell where the results have proved to be correct but the mathematical arguments are faulty.

In 1854 Maxwell was second wrangler and first Smith's prizeman; he was elected to a Fellowship at Trinity, but his father's health was deteriorating, and he wished to return to Scotland. In 1856 he was appointed to the Chair of Natural Philosophy at Marischal College Aberdeen; but unfortunately before the appointment was announced his father died. This was a great personal loss; he had a close relationship with his father, they had corresponded almost daily, and had always spent vacations together.

Maxwell must now have felt the need for his own family life, and in June 1858 he married Katherine Mary Dewar, daughter of the Principal of Marischal College. The union was childless, and is described as a "married life . . . of unexampled devotion". When Maxwell was away from home he would write to his wife every day, sometimes even twice a day, giving all details of his activities. When his wife was ill during their stay in Cambridge he did not sleep in a bed for three weeks. Reciprocally when Maxwell had an attack of smallpox at Glenlair in September 1860 his wife was left quite alone with him—the servants coming only to the door of the sideroom.

Later biographers have been less sympathetic to Mrs Maxwell. However, Everitt points out that the suggestion repeated by many writers (including Sir Edmund Whittaker) that she attempted to interfere with his scientific work, emanated from Mrs Tait, and should be completely discounted. For example, his wife helped him devotedly in his experiments on colour vision and the viscosity of gases.

Maxwell moved from Aberdeen to London in 1860. He took up residence in Kensington where he stayed until 1866. It was here that an experimental test was undertaken of his theoretical prediction that the viscosity of a gas should be independent of its density, his wife acting as stoker. He carried out many other experiments in a large attic which ran the whole length of the house. When he experimented at the window with the colour-box used for studies of colour vision (painted black, and nearly eight feet long) his neighbours thought him crazy to spend so many hours staring into a coffin.

After Maxwell retired to Glenlair, he continued to visit London every spring, and served as external examiner for the Mathematics Tripos at Cambridge. In the spring and early summer of 1867 he toured Italy; he had a good knowledge of French, German and Italian, the only language which gave him any difficulty being Dutch. But most of his energy during this period was devoted to writing his famous treatise on electricity and magnetism.

In 1871 when he was approached regarding the new Chair at Cambridge, Maxwell showed no enthusiasm for the position. In his reply to the Reverend E. W. Blore he said "I had no intention of applying for the post when I got your letter, and I have none now, unless I come to see that I can do some good by it." But after persuasion by G. G. Stokes, Lord Rayleigh and others he allowed his name to go forward and was duly elected.

He set about designing and superintending the erection of the Cavendish Laboratory. The building was not completed until 1874, and was not furnished with proper apparatus until 1877. Maxwell had few students, but they were of the highest calibre, and included W. D. Niven, Ambrose Fleming, R. T. Glazebrook, J. H. Poynting and Arthur Schuster.

At Cambridge Maxwell spent a great deal of time and effort editing the unpublished manuscripts of Henry Cavendish (who had donated the funds for the establishment of the Cavendish

Major contributions to science

Electromagnetism: It is Maxwell's researches on electromagnetism which have classified him with the great scientists of history. Maxwell always acknowledged his debt to Faraday, and in the preface to his *Treatise on Electricity and Magnetism* says that his major task was to convert Faraday's physical ideas into mathematical form, and hence to make them more widely accessible.

ON PHYSICAL LINES OF FORCE.

The velocity of light in air, as determined by M. Fizeau*, is 70,843 leagues per second (25 leagues to a degree) which gives

$$V = 314,858,000,000 \text{ millimetres}$$

$$= 195,647 \text{ miles per second} \dots\dots\dots (137).$$

The velocity of transverse undulations in our hypothetical medium, calculated from the electro-magnetic experiments of M.M. Kohlrausch and Weber, agrees so exactly with the velocity of light calculated from the optical experiments of M. Fizeau, that we can scarcely avoid the inference that *light consists in the transverse undulations of the same medium which is the cause of electric and magnetic phenomena.*

Extract from Maxwell's 1862 paper in the *Philosophical Magazine* in which he first formulated the electromagnetic theory of light.

His original contributions to electromagnetism are contained in three large sets of papers. The first, "On Faraday's Lines of Force", was published in 1856 during his first stay at Cambridge. Its aim was to derive all the standard results of electricity and magnetism using the 'medium' concept rather than that of action at a distance. The second, "On Physical Lines of Force", appeared in 1861–62. In attempting to illustrate Faraday's law of induction (that a changing magnetic field gives rise to an induced e.m.f.) Maxwell constructed a mechanical model and found that there would be a corresponding 'displacement current' in the dielectric medium. This medium could then be the seat of transverse waves, and on calculating the velocity of these waves he found that they were very close to the velocity of light. He concluded dramatically "we can scarcely avoid the inference that light consists in the transverse undulations of the same medium which is the cause of electric and magnetic phenomena". The final paper "A Dynamical Theory of the Electromagnetic Field", published in 1864, dispensed with the mechanical model, and formulated the field equations in the way in which they have been used subsequently.

Colour vision: To understand the theory of colour vision it is important to differentiate between the optical (or true) spectrum of a beam of light and the chromatic spectrum as seen by the eye. Beams of light with different optical spectra can have the same chromatic spectrum. Isaac Newton had shown that the optical spectrum of white light is very complex, but Thomas Young at the beginning of the nineteenth century proposed that any chromatic spectrum can always be analysed into three components corresponding to three receptors in the eye.

Maxwell's work on colour vision started when he was an undergraduate at Edinburgh and continued for many years afterwards. He chose red, green and blue as primary colours, and devised a colour top by means of which tinted papers of different colours could be mixed in varying intensities and the results determined. In this way he initiated 'quantitative colorimetry', analysing every hue into its appropriate components. Maxwell made the important observation that Young's hypothesis works only if negative as well as positive contributions are allowed. He also suggested that colour blindness corresponds to the absence of a red-sensitive receptor, and devised a pair of spectacles to enable a colour blind person to differentiate between red and green.

He invented a colour box in which beams of light of different colours could be mixed in variable proportions to match any given hue. With this apparatus he was able to achieve great accuracy in his experiments to chart the colour response of



The house which the Maxwells occupied during their stay in London (1860–66). The address was then 8 Palace Gardens Terrace, Kensington, but in 1869 the number was changed to 16. Experiments on colour vision and viscosity of gases, in which he was helped by his wife, took place in the attic.

Laboratory). Maxwell did a superb job, repeating key experiments, and establishing priority for Cavendish in the discovery of the inverse square law of electrostatics. But it cost all the spare time of the last five years of his short life, and surely his genius could have been put to more profitable use.

In the Easter term of 1879 Maxwell was ill on several occasions; he returned to Glenlair in June but his condition did not improve. On 2 October he was told that he had no more than one month to live. The religious faith which had characterised his whole life became even more manifest during his last few weeks. He returned to Cambridge, and expressed particular concern that proper arrangements should be made to look after his wife whose health had been poor. His mind remained clear to the end; in the words of Dr Paget who attended him "No man ever met death more consciously or more calmly".

different individuals, and to demonstrate that the colour discrimination of normal people is very good.

Colour photography: From the three-colour theory Maxwell concluded that by photographing through filters of three different colours, and then recombining the images, it should be possible to make a colour photograph of any object. He demonstrated this practically in a lecture to the Royal Institution in 1861 by photographing a tartan ribbon. This demonstration should not have worked, since the photographic emulsions available to Maxwell were not red-sensitive. The mystery was solved when a group of Kodak workers repeated Maxwell's procedure exactly, and found that the red filter also passed UV, and the red in the ribbon apparently also reflected UV.

Saturn's rings: Whilst at Aberdeen Maxwell was able to demonstrate his mastery of classical physics in his Adams Prize Essay on Saturn's Rings, described by George Airy as one of the most remarkable applications of mathematics he had ever seen. Three alternative hypotheses were advanced for the constitution of Saturn's rings: (1) that the rings are solid, (2) that they are fluid or in part uniform, (3) that they consist of masses of matter not mutually coherent; it was necessary to examine for each hypothesis whether the conditions of stability are satisfied by the mutual attractions and motions of the planet and the rings. Maxwell concluded that the third hypothesis was the only one compatible with the stability of the rings. His investigation of this hypothesis laid the foundation for his subsequent work on the kinetic theory of gases.

Kinetic theory of gases

At the British Association meeting in Aberdeen in 1859 Maxwell pointed out that the velocities of molecules in a gas must follow a statistical distribution. Previously it had been naively assumed that they all had the same velocity. Maxwell derived a formula for this distribution at a given temperature and density, which has come to be known as the Maxwellian distribution. Although his derivation contained unwarranted assumptions, his result is valid and represents one of the most widely used formulae in statistical mechanics.

In later papers Maxwell investigated the transport properties of gases—the behaviour of viscosity, thermal conductivity and diffusion with change of temperature and pressure. He concluded that the viscosity of a gas should be independent of pressure, and found this result in reasonable agreement with experiment. He noted a particular simplification which occurs for molecules interacting with a repulsive force inversely proportional to the fifth power of their distance apart. In the last major paper of his life, published in 1879, he laid the foundations of the theory of rarefied gas dynamics.

Fertile ideas

Several casual ideas of Maxwell have led to important developments. Perhaps the best known is the Maxwell Demon which he introduced near the end of his text-book *Theory of Heat*, published in 1871, to demonstrate the statistical character of the second law of thermodynamics. The demon was a hypothetical being who could follow the path of every molecule, and seated on a trapdoor between two vessels A and B, would open and close the door so as to allow fast molecules to go from A to B and slow molecules from B to A. Hence the temperature of B would be increased and A decreased without expenditure of work thereby contravening the second law of thermodynamics. The suggestion intrigued many distinguished scientists subsequently. If one could devise a practical realisation of the demon, the consequences could be very important. Clearly the demon's intelligence must be related in some way to entropy, and this correlation was given a mathematical formulation in Brillouin's theory of information which thereby demonstrated that the demon could not operate. Brillouin's interpretation of information as negative entropy, which was responsible for exorcising the demon, is a key concept in present understanding of the thermodynamics of biological systems.

In 1868, Maxwell provided the first analytical treatment of governors, devices which ensure that the velocity of the machine remains nearly uniform despite variations in driving power or resistance. Devices of this kind had been used by James Watt (to control his steam engine), James Thomson, C. W. Siemens, M. Foucault and others. Maxwell derived differential equations for the motion, introducing a term later to be characterised as negative feedback. He solved the equations, and determined conditions for the motion to be stable, and the governor to function. His paper laid the foundation of control theory, or cybernetics.

The Bakerian Lecture to the Royal Society in 1869 was delivered by Thomas Andrews, and described the classic experiments on carbon dioxide from which he derived the relationship between the liquid and gaseous states. Four years later Johannes van der Waals presented his thesis in which, starting from a plausible molecular hypothesis, he derived his equation of state for fluids. Maxwell was very impressed by this thesis, and made the effort of learning Dutch to be able to study it. He reviewed it in *Nature* in 1874, and in a lecture to the Chemical Society in 1875, where he supplied a crucial step missing in the van der Waals treatment. The argument which Maxwell used to justify his construction was heuristic rather than rigorous; it was only in the 1960s that this result was shown to be rigorously correct.

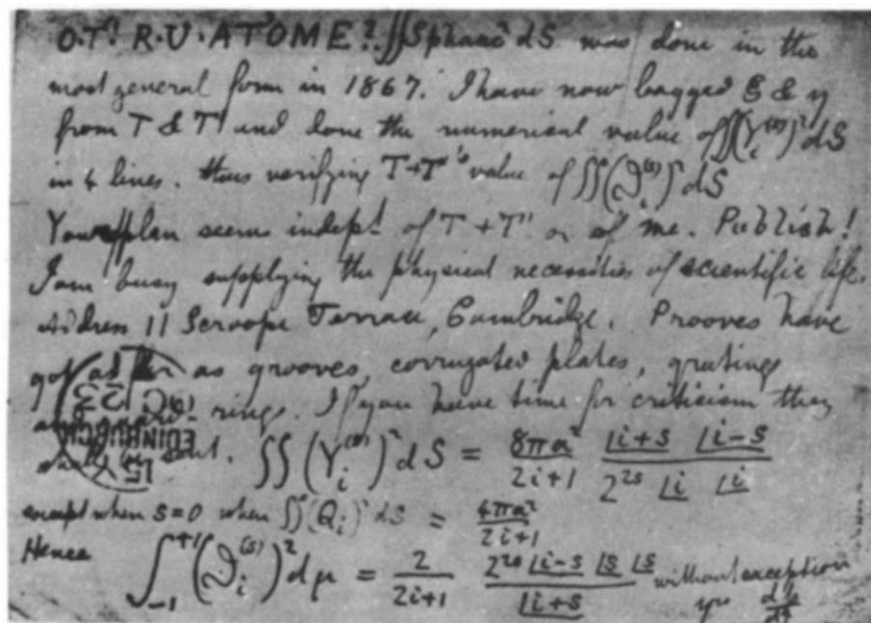
Whilst still a student at Cambridge in 1853 Maxwell undertook a study in geometrical optics "suggested by the contemplation of the structure of the crystalline lens in fish". He looked for a medium in which focusing would be perfect—all rays proceeding from any point in the medium would meet accurately in another point; and using a method deduced by analogy from Newton's *Principia* he arrived at a mathematical description of the medium. Nearly a century elapsed before Rudolf Luneberg revived the subject, and found other instances of perfect imaging devices.

A striking example of the casual way in which Maxwell produced original ideas is provided by the theory of propagation of light in a dispersive medium. A formula for the refractive index was put forward by Sellmeyer in 1871. Twenty years after Maxwell's death, Lord Rayleigh published a note in the *Philosophical Magazine* pointing out that Maxwell had considered the question before anyone else. But Sellmeyer could hardly have been expected to know of Maxwell's work as it was contained in a question set for the Mathematical Tripos examination on 21 January 1869.

Photographed by R. A. R. Tricker



Glenlair, the country house which Maxwell inherited and to which he moved when he resigned from King's College in 1865. He left in 1871 to become the first Cavendish professor at Cambridge. (The interior of the house was destroyed by fire in 1929.)



One of the postcards sent by Maxwell to his friend Peter Guthrie Tait. William Thomson (Lord Kelvin) is referred to as T, P. G. Tait as T', and Maxwell signs himself dp/dT in accordance with the thermodynamic relation $dp/dT = JCM$. The term T & T' used in lines 3 and 4 refers to the famous nineteenth century text by Thomson and Tait, *Elements of Natural Philosophy*.

Maxwell and Einstein

Maxwell regarded the ether as the seat of electromagnetic waves, and after developing his electromagnetic theory, he devoted much thought to the problem of the 'ether drift' or how much the velocity of light would be changed by the motion of the emitter. He convinced himself that in a terrestrial experiment it was necessary to measure the time which has elapsed in a go and return journey and the effect would be of the order of v^2/c^2 (where v is the velocity of the emitter and c the velocity of light) which would be too small to measure. But in the last year of his life he wrote to the astronomer D. P. Todd suggesting that he look for a change in the periodicity of the satellites of Jupiter as the planet changed its position relative to the Earth; this periodicity should depend on the ether velocity along a line joining the Earth and the planet. These considerations were an important stimulus to the famous Michelson-Morley experiment which played such a crucial role in Einstein's development of special relativity.

Maxwell maintained a close scientific friendship with William Thomson (later Lord Kelvin) and Peter Guthrie Tait. For many years the three exchanged postcards, the former being referred to as T and the latter as T', and Maxwell as dp/dT (this was based on the thermodynamic relation $dp/dT = JCM$ giving Maxwell's initials, where p is the pressure, T the temperature, J Joule's constant, C Carnot's function T^{-1} , and M the rate of increase of heat in an isothermal change of volume). One of the most influential text books of the period was Thomson and Tait's *Treatise on Natural Philosophy*. When Tait sent a preliminary draft to Maxwell he wrote back criticising them for confusing mass and weight: "You may place the two masses in a common balance (which proves their weight equal), you may then cause the whole machine to move up or down. If the arm of the balance moves parallel to itself the masses must also be equal."

The identity of mass-like and weight-like properties of matter is a coincidence in Newton's theory, and constituted one of Einstein's major criticisms of the theory.

In his classic 1864 paper "A Dynamical Theory of the Electromagnetic Field" Maxwell tried to consider the formulation of a field theory of gravity which also satisfies an inverse square law of force. But the change of sign in the interaction and the requirement that energy must be positive led to the conclusion that an enormous intrinsic energy must be associated with the medium. "As I am unable to understand in what way a medium can possess such properties I cannot go further in this direction in searching for the cause of gravitation." Maxwell clearly wanted to go in the Einstein direction, but the necessary basic equipment was lacking.

Finally in a lecture delivered to the Chemical Society in 1875 Maxwell calculated the specific heat of a gas of molecules with n degrees of freedom. He found that the ratio γ of C_p the specific heat at constant pressure, to C_v the specific heat at constant volume, should be of the form $(2+n+e)/(n+e)$, where e is a positive quantity of unknown value relating to the stored energy of the molecule. For a monatomic molecule $n=3$, $e=0$ (no stored energy), $\gamma=5/3$, a value which agrees well with experiment. For a diatomic molecule $n=6$, and the largest possible value of γ (corresponding to $e=0$) is 1.33; but the experimental values for several gases are found to be 1.408. Maxwell continued "I have now put before you what I consider to be the greatest difficulty yet encountered by the molecular theory".

The difficulty was resolved only by Planck's quantum hypothesis; and it was Einstein who first applied the hypothesis to the calculation of specific heats, and showed that the energy of an oscillator became frozen at sufficiently low temperatures.

Maxwell's versatility

This record by no means exhausts Maxwell's contributions to science and to general culture. This would require a much larger and more comprehensive article. He showed considerable ability as a poet, particularly for semi-humorous verse on scientific themes. He was concerned with scientific terminology, for example, coining the term curl which is now used almost universally in vector analysis. Amongst other scientific innovations he produced a theorem on reciprocal diagrams in frameworks which he later extended to continuous media, and which was of importance in the development of ideas on strength of materials. He devoted much time and effort trying to popularise the Gibbs approach to thermodynamics.

Maxwell's papers are full of novelty and interest. In 1854 he discusses what happens when a slip of paper falls through the air, noting that its general path is not in the vertical direction but inclined to it at an angle which remains nearly constant, its fluttering appearance due to a rapid rotation around a horizontal axis. He then advances reasons for this behaviour.

In the words of the late Charles Coulson "there is scarcely a single topic that he touched upon which he did not change almost beyond recognition".

I am indebted to the Syndics of the Cambridge University Library for permission to reproduce the illustrations indicated; and to Pergamon Press Ltd for permission to reproduce the photograph of Glenlair from *The Contributions of Faraday & Maxwell to Electrical Science* by R. A. R. Tricker.

articles

An absorption feature in the spectrum of the pulsed hard X-ray flux from 4U0115+63

Wm. A. Wheaton, J. P. Doty, F. A. Primini, B. A. Cooke, C. A. Dobson, A. Goldman, M. Hecht, J. A. Hoffman*, S. K. Howe, A. Scheepmaker†, E. Y. Tsiang & W. H. G. Lewin

Department of Physics and Center for Space Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

J. L. Matteson, D. E. Gruber, W. A. Baity, R. Rothschild, F. K. Knight, P. Nolan & L. E. Peterson

Physics Department, University of California at San Diego, La Jolla, California 92093

A spectral feature, apparently an absorption line, has been observed at an energy of 20.1 ± 0.5 keV in the pulsed flux of 3.61 s X-ray pulsar 4U0115+63 using the UCSD/MIT instrument on HEAO 1. The line strength, expressed as equivalent width, is 3.1 ± 0.5 keV. Although essentially unresolved, the feature has a depth more than 60% of the continuum flux. If the feature arises by cyclotron resonance absorption near the magnetic poles of the neutron star, it implies a magnetic field of between ≈ 1.8 and $\approx 2.5 \times 10^{12}$ G depending on the gravitational redshift (≈ 5 –40%).

exclude the possibility that the observed phenomenon results from some quite different cause.

Observations

HEAO 1 pointed at 4U0115+63 (ref. 17) on 16 January 1978, when the source was near its peak brightness, for ≈ 3 h centred at ≈ 15.00 UT. Each of the two low energy detectors (LED1 and LED2) of the UCSD/MIT hard X-ray and low energy γ -ray experiment¹⁸ had a ≈ 0.3 cm thick, actively shielded NaI 'phoswich' scintillation detector of area ≈ 100 cm² collimated to a $1.2^\circ \times 23^\circ$ (FWHM) field-of-view by slat collimators with an energy resolution of ~ 6 keV (FWHM) at 20 keV. X-ray events in the 12–180 keV range are analysed in the 64-channel linear pulse height analyser (PHA) and transmitted event-by-event over a single telemetry channel shared among seven detectors. The mean residence time of events in the telemetry buffers determines the time resolution of about 0.05 s, binned here to 0.32 s. After elimination of Earth-blocked data and correction for electronic and telemetry dead times, each detector yielded about 6,000 s of useful data.

Fourier transforms and folds gave a best geocentric period of 3.61361 s during the observation, consistent with the SAS 3 ephemeris¹². Figure 1 shows the data from LED2, folded modulo the pulse period and summed over the 12–66 keV interval in which we observe a significant pulsed flux. Comparison of this 'template' pulse shape with the data in individual PHA channels (Fig. 2) shows no strong variation of pulse profile with energy, but a marked reduction in pulse amplitude in the 19.3–24.8 keV band. In these circumstances it becomes possible to discuss the data in terms of a simple two-component model, in which we represent the observations as a sum of pulsed and steady fluxes. To determine the spectrum of each of these two components we fit the folded data in each PHA channel by the sum of two functions of the pulse phase—the first a constant, and the second given by the template. The smooth curves of Fig. 2 show the resulting fits. These smooth curves all have the same shape and phase alignment, which are those of the template. Only their amplitudes have been adjusted to fit the data. Because of minor differences in time and energy resolution and gain between the two detectors, we fit each independently, obtaining the spectra shown in Fig. 3.

ALTHOUGH standard models (see ref. 1) of the pulsating binary X-ray sources as rotating, accreting magnetised neutron stars require magnetic fields B of the order of 10^{12} – 10^{13} G, the discovery by Trümper *et al.*² of a strong pulsed 58 keV feature in 1976 and 1977 balloon observations of the spectrum of Her X-1 raised the important possibility of direct measurements of B . Trümper *et al.*² have argued that the feature they observed may result, as suggested earlier on theoretical grounds by Basko and Sunyaev³, from cyclotron emission quantised in a field of $\approx 5.3 \times 10^{12}$ G (uncorrected for redshift). The UCSD/MIT experiment on HEAO 1 confirmed the existence of this feature in the spectrum of Her X-1 on several occasions in 1978 (refs 4, 5).

The 1978 outburst^{6,7} of 4U0115+63 led to discovery of its 3.61 s pulsations^{6–8}, accurate measurement of its position^{9,10}, and discovery of its binary nature¹¹, 24-d orbit¹², and 15th mag B-type optical counterpart¹³, as well as the determination of X-ray spectra and pulse profiles from 0.9 to 40 keV^{14,15}. Here we present details of the evidence for the 20 keV feature previously reported¹⁶, but defer a complete discussion of the 12–180 keV spectral and temporal behaviour of the source. We caution that despite the attractiveness of the cyclotron interpretation of the feature, pending better understanding of the complex physics of radiation transfer in the magnetised, semi-relativistic atmosphere of an accreting neutron star, we cannot

* Permanent address: Astronaut Office, NASA Johnson Space Center, Houston, Texas 77058.

† Permanent address: Cosmic Ray Working Group, Leiden, The Netherlands.

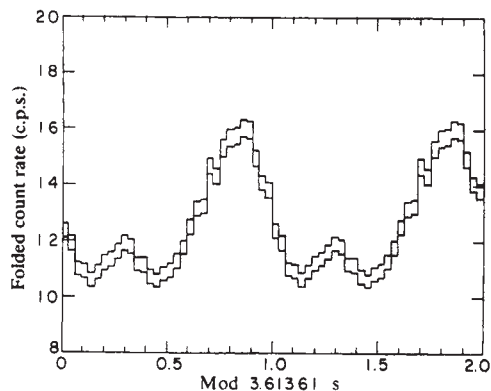


Fig. 1 Counting rates (12–66 keV) in low energy detector 2 (LED2) folded modulo the pulse period. LED1 shows a virtually identical profile. The upper and lower histograms show the data $\pm 1\sigma$, for two complete pulse periods. The data at any energy from 12 to 66 keV can be well represented by a sum of constant and a pulse of this shape. The plot includes a background count rate of 3.7 counts per second (c.p.s.). Phase 0 refers to JD 2443525.1134055.

The two lower sets of points (Fig. 3) show the pulsed spectrum in each detector, where we define the pulsed flux as the excess flux above the minimum of the fitted pulse profile, averaged over one 3.61 s cycle. Because the background is unpulsed, these spectra required no background subtraction, and their error bars reflect only counting statistics. This also allowed us to include data with the Earth's horizon in the field of view, which increases the useful exposure from the $\approx 3,800$ s included in the total spectrum (and in our preliminary report¹⁶) to $\approx 6,000$ s.

The large dip in the pulsed LED2 spectrum in PHA channels 9 and 10 corresponds to the low amplitude of pulsation noted in the 19.3–24.8 keV band of Fig. 2. The smaller dip in LED1 results partly from poorer resolution (since the feature is essentially unresolved—see below) in that detector, $\approx 33\%$ (FWHM at 20 keV) as against $\approx 29\%$ for LED2, and, we must conclude, partly from a statistical fluctuation. By a series of detector response calculations, detailed below, we have established that the combined probability (taking both detectors into account) that the observed feature could have arisen by a statistical fluctuation from a smooth continuum is very small ($\chi^2 = 50$ for $\nu = 7$ d.f.) but that adding a narrow absorption line to the model gives an improved χ^2 of 9.6 ($\nu = 5$) for the model of best fit. Because of the difference (in the depth the feature) between the two detectors, more complicated model photon spectra cannot be expected to improve χ^2 very much, so we accept the fit. For the model of best fit we obtained an absorption line energy $E_0 = 20.1 \pm 0.5$ keV and a line strength, expressed in terms of the equivalent width of continuum absorbed, of $\Delta E = 3.1 \pm 0.5$ keV. Independent fits to each detector separately yielded $\Delta E = 2.0 \pm 0.7$ keV for LED1 and $\Delta E = 4.1^{+1.1}_{-0.7}$ keV for LED2. Such an absorption line must have a depth exceeding 0.6 of the continuum level (95% confidence), which corresponds to a maximum actual width of about 5 keV ($= 3.1 \text{ keV}/0.6$). Systematic uncertainties, principally due to the idealised two-component analysis and to imperfections in the detector response models, may increase the absolute errors for E_0 and ΔE from the purely statistical errors quoted.

Experiments with different methods of analysis from those reported here, and with reasonable modifications of the detector response models, lead us to estimate the maximum likely increase in these errors to be less than a factor of two.

In these calculations we used response models for the two detectors based on pre-launch calibrations. We compared the expected counts for given input photon spectra with the actual data by a χ^2 test which treated the two detectors as a single experiment, summing χ^2 over the data from the five PHA channels nearest the feature in each detector. Systematic errors in the HEAO 1 attitude determination during the observation

required independent normalisations for LED1 and LED2. Since we are concerned here only with the immediate vicinity of the feature, it was sufficient to use a simple form for the continuum, and we chose $dN/dE = A \exp(-E/E_c)$. For the hypothesis of no feature we fitted for the continuum slope E_c and the two normalisations, obtaining $\nu = 7$, and for the line hypothesis fits we added E_0 and ΔE , obtaining $\nu = 5$. We determined the single parameter, 1σ statistical errors in E_0 and ΔE , and the constraint on the line depth, using criteria discussed by Avni¹⁹.

As the feature appears in the 3.61 s pulsed spectrum, it is very difficult to understand as an artefact of the instrument or analysis. It appears neither in the background nor in the unpulsed spectrum of 4U0115+63, nor in any other source spectra observed with the same instrument and analysed in the same way, whether pulsed (such as Her X-1, GX1+4, 4U0900–40 and 4U1626–67)^{4,5,18} or total (Crab Nebula, Cyg X-1). We have divided the data into two halves, and the dip appears in each of them.

The two-component analysis which leads to the pulsed spectra of Fig. 3 has the important feature that, in the language of signal processing, it filters the data with the expected signal shape (the template), which yields optimal sensitivity. However, if there is a strong variation with energy of pulse profile or phasing this

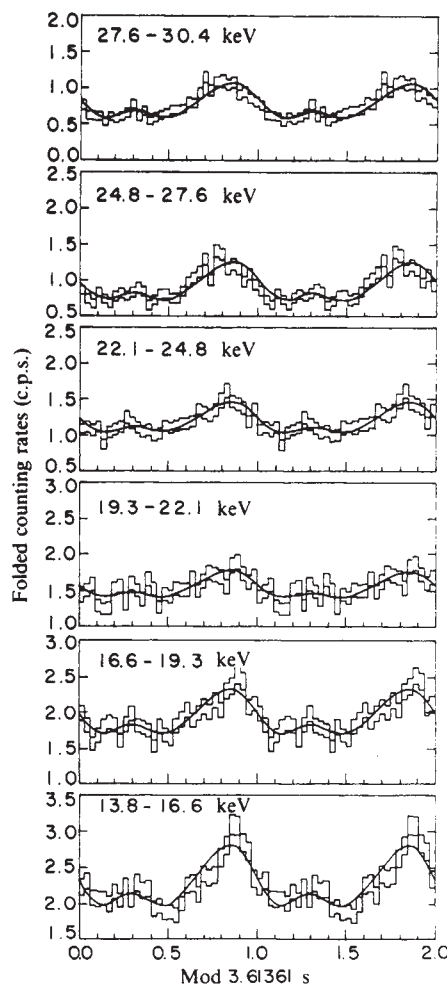


Fig. 2 Folded counting rates from LED2 in PHA channels 7–12, displayed similarly to Fig. 1, all to the same scale. The 19.3–24.8 keV channels (PHA 9 and 10) are noticeably less strongly pulsed than adjacent channels, possibly due to cyclotron resonance absorption. The continuous curves show fitted pulse profiles derived as described in the text, only smoothed for clarity by omission of all but the first three Fourier frequencies. Background rates for PHA channels 7 to 12 of 0.48, 0.37, 0.30, 0.26, 0.23 and 0.24 (c.p.s.), respectively, are included.

analysis could introduce spurious features. Data taken simultaneously by the Goddard Space Flight Center experiment on HEAO 1 show rapid changes in overall pulse shape with energy below about 10 keV (ref. 21) but examination of the data above 13 keV and the fits in Fig. 2 shows only small changes in pulse profile, which do not change the general shape or phase of the pulse, even in the channels with the dip, and do not account for the reduction in pulse amplitude there. A χ^2 test of the data in each PHA channel against the hypothesis of no pulsation gave $\chi^2 = 90$ (for 31 d.f.) in the channel with the dip, but $\chi^2 = 150$ –190 at both higher and lower energies, which establishes the reality of the feature independent of any assumptions about pulse shape. Pravdo *et al.*²² pointed out that incautious subtraction of physically different 'on-pulse' and 'off-pulse' spectra could produce an apparent absorption feature with no underlying physical significance. While one can never rigorously exclude such an explanation, the deep and narrow feature observed make it artificial in this case. We observe a pulse that varies strongly in amplitude but very little in shape as a function of energy. For such behaviour the picture of independent emission spectra with pulse phase offers no natural interpretation.

The two upper sets of data in Fig. 3 show the total (pulsed plus unpulsed) spectrum in each detector. In the analysis of these total spectra we used only data in which the Earth lay completely outside the collimator field of view in order to avoid large systematic background variations. We estimated the background from $\approx 4,000$ s of sky exposure in each detector obtained in eight roughly equal intervals from the two orbits of scanning data adjacent to the observation; the errors shown for the total spectra include the effects of systematic variations in the background as estimated from the scatter among these eight intervals. Figure 4 shows the photon spectrum for LED2 corresponding to the total count spectrum of Fig. 3b, derived by dividing by detector efficiencies determined from the instrument

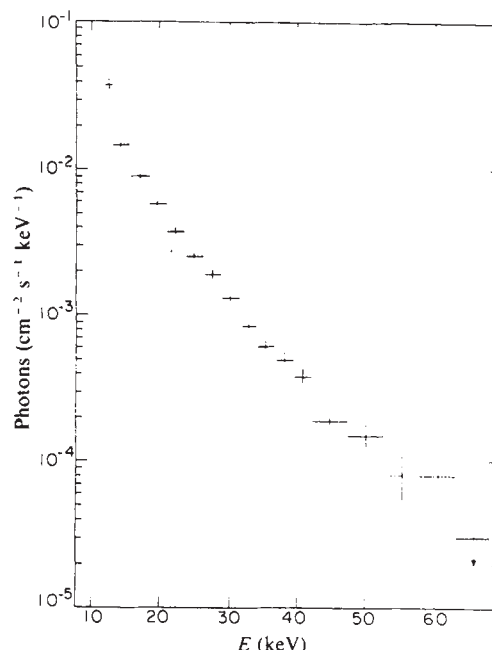


Fig. 4 Photon spectrum of the total flux (pulsed plus unpulsed), using the data from Fig. 3b, and based on a calibration using the Crab Nebula.

response to the Crab Nebula. The spectrum is close to a power law with number spectral index ≈ 3.9 or to $\approx 10^8$ K thermal bremsstrahlung. A correction of 0.85 for the collimator transmission averaged over small aspect variations during the point was applied.

Discussion

Our results are consistent with all the other data known to us. However, the UCSD/MIT observation obtained about an order of magnitude more counts in the 20 keV range than any other instrument of comparable energy resolution. Hence no detailed confirmation of the 20 keV feature seems likely until further observations can be obtained.

Figure 3 shows that the pulse fraction, defined here as the ratio (pulsed flux)/(total flux), decreases from $\approx 50\%$ at 60 keV to $\approx 17\%$ at 15 keV. Johnston *et al.*¹⁴ have found that it continues to decline to below 2.5 keV. Because the pulsed spectrum is harder than the total spectrum, the spectrum must harden near the peak of the pulse, behaviour generally similar to that seen from 0.9 to 18 keV by others^{14,15}. However, the main peak observed below about 10 keV occurs at a different phase, about 0.5 instead of our 0.85.

Note that we use the term 'absorption' to include 'cyclotron resonance scattering'. The concavity of the total flux (Fig. 4) near 20 keV is sufficient to accommodate either absorption, which would entirely remove photons, or scattering, which could merely redistribute them in direction and thus pulse phase. However, as scattered photons could be beamed in directions never seen by Earth-bound observers, the two are essentially indistinguishable observationally.

The results for Her X-1 due to Trümper *et al.*² can be interpreted as absorption at energies below ≈ 42 keV as well as emission at ≈ 58 keV. The data presented here, because of the deep and narrow feature, favour absorption/scattering as the correct interpretation for 4U0115+63 but cannot rule out an emission line explanation, in which the broad (about twice the instrument energy resolution) hump around 28 keV in the pulsed spectrum results from an emission blend seen over a steeply falling continuum. If due to cyclotron emission, our data would indicate a correspondingly higher and less uniform magnetic field. As we expect a cutoff in cyclotron emission corresponding to the maximum surface field near the magnetic

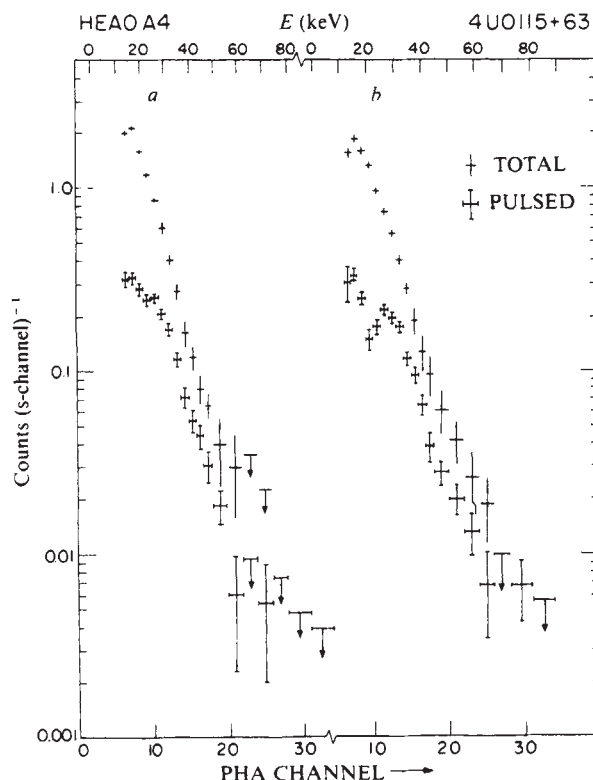


Fig. 3 Pulse height spectra of the total (upper) and pulsed (lower) counts in: a, LED1; and b, LED2. The 20 keV feature shows most clearly in LED2 in PHA channel 9. If it results from cyclotron resonance absorption, it corresponds to a surface magnetic field B of ~ 1.8 – 2.5×10^{12} G, (depending on the gravitational redshift), probably near the poles of the neutron star.

poles, the observed extension of the pulsed flux above the feature suggests an additional hard component of the continuum. No full theoretical treatment predicting the relative likelihood of cyclotron emission or absorption/scattering has yet appeared, though each has been briefly considered in the literature^{3,23,24}. Dimensional estimates tend to support scattering/absorption over emission (F. Lamb, personal communication). Clear evidence for higher (or lower) harmonics could settle this question, but it does not appear in these data.

Assuming that the 20 keV feature arises near the surface of the neutron star, a gravitational redshift of ≈ 5 –40% (ref. 25) gives 20–28 keV as the surface energy corresponding to a 20 keV line. If, following Trümper *et al.*² we interpret this as due to cyclotron absorption from the ground state ($j=0, s=-1$) to the first excited state ($j=1, s=-1$) or ($j=0, s=+1$) and assume negligible change in the electron momentum parallel to the magnetic field B , we obtain $B \approx (1.8 \text{ to } 2.5) \times 10^{12}$ G, probably near the base of the accretion column near the magnetic pole. The narrowness of the line implies it must arise in a region of fairly uniform field, since $\Delta B/B \approx \Delta E/E \approx 0.25$. Because the radial extent Δr of the absorbing region must satisfy $\Delta r/r \leq \frac{1}{2}(\Delta B/B)$ (ref. 2), if $r \approx 10$ km, then Δr must be < 1 km. Since redistribution of photons in energy would tend to fill in a narrow

absorption line, its width and strength limit the amount of reprocessing matter that can lie along the line of sight. Thus, this source does not seem to be observed through a cold magnetospheric shell of the type proposed for Her X-1 (refs 26, 27), as discussed in detail by Weaver²⁸.

As we have already constrained the height of the emission region to < 1 km, any future improvements in resolution will begin to show whether it is more like the size of the accretion hot spot (≈ 1 km) or the atmospheric scale height (≈ 1 m). Thermal motions along the magnetic field line should also contribute to the linewidth. Since this effect depends on the cosine of the angle between the field and the line of sight, observation of the broadening as a function of pulse phase could eventually give both the temperature of the absorbing electrons and the geometry of the system.

We thank members of the HEAO project and the people in the HEAO Operations Control Center for their work on making the pointed observation successful. We also thank L. A. Rose and S. Pravdo for comments and for results from the GSFC experiment prior to publication, and J. Trümper, L. Cominsky, J. McClintock, B. Rossi and J. van Paradijs for helpful comments. This work was supported by NASA contracts 8-27974 and 8-27975.

Received 10 May; accepted 31 August 1979.

- Lamb, F. K. *Ann. N.Y. Acad. Sci.* **302**, 482 (1977).
- Trümper, J., Pietsch, W., Reppin, C. & Voges, W. *Astrophys. J. Lett.* **219**, L105–L110 (1978); *Ann. N.Y. Acad. Sci.* **302**, 538 (1977).
- Basko, M. M. & Sunyaev, R. A. *Astr. Astrophys.* **42**, 311–321 (1975).
- Matteson, J. L., Gruber, D. E. & Hoffman, J. A. in *Gama Ray Spectroscopy in Astrophysics* **386**, (NASA TM 79619, 1978).
- Gruber, D. E. *et al. Bull. Am. astr. Soc.* **10**, 506 (1978).
- Holt, S. S. & Kaluzienski, L. J. *IAU Circ. No.* 3161 (1978).
- Clark, G. & Cominsky, L. *IAU Circ. No.* 3161 (1978).
- Cominsky, L., Clark, G. W., Li, F., Mayer, W. & Rappaport, S. *Nature* **273**, 367–369 (1978).
- Cominsky, L., Li, F., Bradt, H., Clark, G. W., & Rappaport, S. *IAU Circ. No.* 3163 (1978).
- Johnston, M. *et al. IAU Circ. No.* 3163 (1978).
- Rappaport, S., Clark, G., Cominsky, L., & Li, F. *IAU Circ. No.* 3171 (1978).
- Rappaport, S., Clark, G. W., Cominsky, L., Joss, P. C. & Li, F. *Astrophys. J. Lett.* **224**, L1–L4 (1978).
- Johns, M., Koshi, A., Canizares, C. & McClintock, J. *IAU Circ. No.* 3171 (1978).
- Johnston, M. *et al. Astrophys. J. Lett.* **223**, L71–L73 (1978).
- Rose, L. A. *et al. preprint* (1978).
- Wheaton, W. A. & HEAO A4 Group, *IAU Circ. No.* 3238 (1978).
- Forman, W., Jones, C. & Tananbaum, H. *Astrophys. J. Lett.* **206**, L29–L35 (1976).
- Matteson, J. L. *Proc. AIAA 16th Aerospace Sci. Meet.*, Huntsville, Alabama, pap. 78–35, (1978).
- Avni, Y. *Astrophys. J.* **210**, 642–646 (1976).
- Doty, J. *et al. Bull. Am. astr. Soc.* **10**, 507 (1978).
- Pravdo, S. H. *et al. Bull. Am. astr. Soc.* **10**, 446 (1978).
- Pravdo, S. H. *et al. preprint* (1978).
- Ginedin, Y. N. & Sunyaev, R. A. *Astr. Astrophys.* **36**, 379–394 (1974).
- Elsner, R. F. & Lamb, F. K. *Nature* **262**, 356–360 (1976).
- Arnett, W. D. & Bowers, R. L. *Astrophys. J. Suppl.* **33**, 415 (1977).
- McCrack, R. & Lamb, F. K. *Astrophys. J. Lett.* **204**, L115–L118 (1976).
- Basko, M. M. & Sunyaev, R. A. *Mon. Not. R. astr. Soc.* **175**, 395 (1976).
- Weaver, R. P. *Nature* **274**, 571–572 (1978).

Plasma acceleration at the Earth's magnetopause: evidence for reconnection

G. Paschmann, B. U. Ö. Sonnerup*, I. Papamastorakis, N. Sckopke & G. Haerendel

Max-Planck-Institut für Physik und Astrophysik, Institut für extraterrestrische Physik, 8046 Garching, FRG

S. J. Bame, J. R. Asbridge & J. T. Gosling

University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87545

C. T. Russell & R. C. Elphic

University of California at Los Angeles, Institute of Geophysics and Planetary Physics Los Angeles, California 90024

A characteristic of magnetic field reconnection is the acceleration of plasma as it flows across a rotational discontinuity. At the Earth's magnetopause this effect has only been observed recently during a few magnetopause crossings by the ISEE satellites. For one example analysed in detail the results show good agreement with theoretical predictions.

MAGNETIC reconnection or merging is thought to have a fundamental role in the conversion of energy stored in magnetic fields, and may occur in many cosmic situations. The concept of reconnection was introduced into magnetospheric physics by Dungey¹ who proposed that reconnection should be operative at

the dayside boundary of the Earth's magnetic field, the magnetopause, as well as in the geomagnetic tail. A detailed description of the process was first provided by Petschek and coworkers^{2,3} who demonstrated that reconnection may occur at substantial rates and that it leads to high-speed plasma jetting away from the reconnection site. Jets of plasma possibly associated with reconnection have been observed in the tail^{4,5} but never at the magnetopause. This negative result has added to the controversy over the nature and efficiency of the reconnection process, despite substantial indirect evidence for its occurrence⁶. Recent measurements from the dual-satellite International Sun–Earth–Explorer Mission⁷ (ISEE) have now shown the occasional presence of high-speed plasma at the magnetopause. We report here on one of these cases in which the observations agree in considerable detail with theoretical predictions.

The basic reconnection configuration for the magnetopause^{3,8} is illustrated in Fig. 1. In this model, the magnetopause is a standing Alfvén wave emerging from the X-shaped centre of the

* Permanent address: Dartmouth College, Radiophysics Laboratory, Hanover, New Hampshire 03755.

reconnection site, referred to as the diffusion region. The magnetic fields on the two sides of the magnetopause current layer, which separates the magnetosheath and terrestrial fields, are not entirely tangential to that layer, but are connected and consequently have a small normal magnetic field component B_n . The magnetosheath plasma flows towards and across the magnetopause, or equivalently, there is a tangential electric field E_t . As the plasma crosses the current sheet, its momentum changes abruptly under the action of the tangential components of the Maxwell stresses. Picturing the sharply bent field lines at the magnetopause as elastic bands acting on the plasma, the process may be likened to a magnetic slingshot, which ejects the plasma at high speed in poleward-directed jets just inside the magnetopause. The energy added to the plasma in this manner is extracted from the electromagnetic field at the rate $E_t \cdot I$, where I is the total magnetopause current.

Observations

Figure 2 shows plasma and magnetic field observations which demonstrate the behaviour described above. The plasma data were obtained with the LASL/MPE quadrispherical analysers¹⁰, which measure the three-dimensional distribution function of ions and electrons with very good time resolution. Full distributions are obtained in three spacecraft revolutions, or

Fig. 1 Reconnection configuration for southward-directed external magnetic field. Solid lines denote magnetic field lines. The dashed line marks the magnetopause, and the shaded area the boundary layer. Magnetosheath and magnetosphere are located to the left and right of the magnetopause, respectively. The normal magnetic field component B_n is negative at the ISEE orbit.

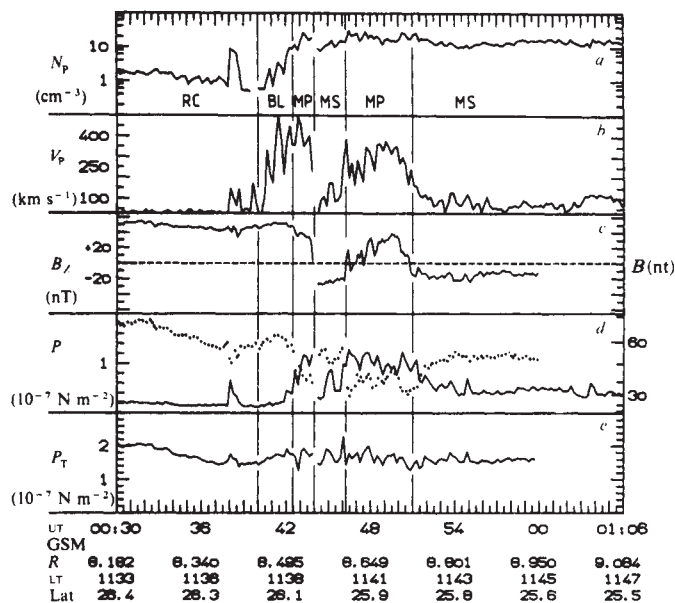
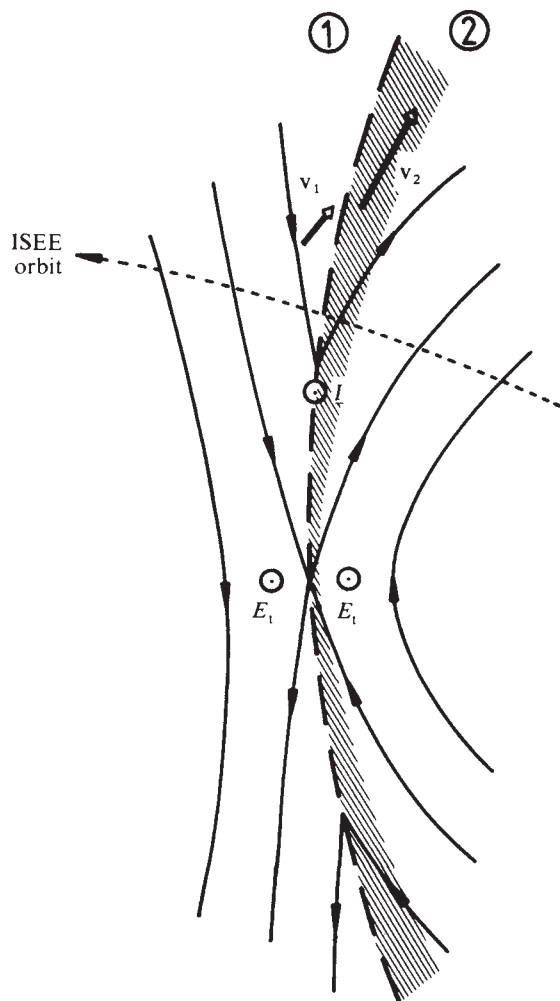


Fig. 2 Plasma and magnetic field data for the ISEE 1 magnetopause crossings on 8 September 1978. *a*, *b* and *c* show the proton density N_p , the proton flow speed v_p , and the magnetic field component B_z , perpendicular to the ecliptic. In *d* the solid line indicates the plasma pressure P , defined as $N_p k(T_p + T_e)$, where k is the Boltzmann constant and T_p and T_e are the proton and electron temperatures, respectively. The dotted line represents the magnetic field strength B , when using the scale on the right, or the magnetic field pressure $B^2/2\mu_0$ with the scale on the left. *e*, The total pressure $P + B^2/2\mu_0$. Plasma parameters are spaced 12 s apart, magnetic field quantities 8 s apart. During the 36 min shown, ISEE 1 moves from a radial distance, R , of 8.2 to 9.1 Earth radii. The local time, LT, is slightly pre-noon throughout the period, the latitude, Lat, stays near 26° (all coordinates in the geocentric solar magnetospheric, GSM, system). Initially ISEE 1 is located in the outer magnetosphere or ring current (RC) region. It then traverses a thin boundary layer (BL) of magnetosheath-like plasma. Due to boundary motions there are multiple magnetopause (MP) encounters before the satellite exits into the magnetosheath (MS). The magnetopause encounters are identified by the transitions in B_z from northward (positive) in the magnetosphere to southward in the magnetosheath.

~ 9 s, and repeated every 12 s. This time resolution which is absolutely necessary in magnetopause studies, is achieved at the expense of energy/angle resolution. For ions, measurements are made over an energy range between 70 eV and 40 keV per charge in 16 channels, covering elevation angles between $+55^\circ$ and -55° in four segments, and 360° of azimuth in eight and four equidistant sectors for the inner and outer elevation segments, respectively. The plasma data are shown as moments of the measured distribution functions¹¹. Data from the UCLA triaxial fluxgate magnetometer¹² are presented here as 12-s averages overlapped by two-thirds so that there is one sample every 4 s.

The data shown in Fig. 2 are from an outbound traversal of the subsolar magnetopause region at a latitude of $\sim 25^\circ$. If we assume that the diffusion region of the reconnection configuration was located near the subsolar point, the measurements were made along a path indicated in Fig. 1. Magnetopause (MP) encounters are identified by the changes in field orientation (B_z panel). Due to motions of the magnetopause, the first, complete crossing is followed by another, incomplete one, before the satellite finally exits into the magnetosheath (MS).

A magnetopause speed of 10 km s^{-1} relative to the spacecraft is derived for the first crossing from the time difference between the ISEE 1 and ISEE 2 crossings, the latter spacecraft being 1,820 km further away from the Earth. Using 10 km s^{-1} , a magnetopause thickness of 900 km is deduced, which is large compared with an ion gyro radius ($\sim 100 \text{ km}$). Figure 2*a* shows that the plasma number density, N_p , was within a factor of 1.5 of the average magnetosheath value during the magnetopause encounters. However, earthwards of the inner edge of the first

crossing, the density dropped gradually to the low values of the outer magnetosphere. This period, denoted BL, corresponds to the low-latitude plasma boundary layer^{13,22}. The density peak within the magnetosphere presumably represents an early partial entry into the boundary layer.

The plasma pressure P and the magnetic field magnitude B show substantial variations during the magnetopause crossings. However, as Fig. 2e shows, the total pressure $P_T = P + B^2/2\mu_0$ remained approximately constant as expected.

The most significant feature shown in Fig. 2 is the extremely large plasma flow speed, V_p , observed during each magnetopause encounter and persisting throughout most of the boundary layer. These high speeds, which were also seen by ISEE 2, peak at 450 km s^{-1} , whereas values in the adjacent magnetosheath fall in the range $50\text{--}100 \text{ km s}^{-1}$. The latter speeds are consistent with the prediction of gas-dynamic models for this location¹⁴. As already mentioned, enhanced flow speeds at the magnetopause are characteristic of reconnection.

Comparison with theoretical expectations

We now compare the observations quantitatively with the predictions of the reconnection model of the dayside magnetopause^{3,8}. In this model the magnetopause is described as a rotational discontinuity—a current layer which possesses a normal magnetic field component and across which the tangential field changes direction by an arbitrary angle. For such a sheet the following relations hold¹⁵

$$\mathbf{v}_{t2} - \mathbf{v}_{t1} = \pm((1 - \alpha_1)/\mu_0\rho_1)^{1/2}[(\rho_1/\rho_2)\mathbf{B}_{t2} - \mathbf{B}_{t1}] \quad (1)$$

$$\rho_2/\rho_1 = (1 - \alpha_1)/(1 - \alpha_2) \quad (2)$$

$$v_n = \pm B_n((1 - \alpha)/\mu_0\rho)^{1/2} \quad (3)$$

$$\alpha \equiv (P_{\parallel} - P_{\perp})\mu_0/B^2 \quad (4)$$

Here $\mathbf{B}$, $\mathbf{v}$ and ρ are the magnetic field vector, plasma flow velocity and mass density, respectively, and the subscript pairs (n, t) and (1, 2) refer to components normal (n) or tangential (t) and exterior (1) or interior (2) to the magnetopause surface; $P_{\parallel}$ and $P_{\perp}$ are the pressures parallel and perpendicular to the magnetic field, respectively, and α is the pressure anisotropy factor. Equation (3) shows that the plasma flows across the sheet with the modified Alfvén speed. Thus, a rotational discontinuity is in fact a large amplitude Alfvén wave. Density changes across such a wave occur only when the anisotropy factor changes, as indicated by equation (2). Finally, equation (1), multiplied by ρv_n , expresses the balance of tangential stresses in the current sheet. The left-hand side is the rate of change of tangential momentum of the plasma as it flows across the layer while the right-hand side, using equations (2) and (3), is seen to represent the sum of the net tangential Maxwell stress $\tau_M = (B_n/\mu_0)(\mathbf{B}_{t2} - \mathbf{B}_{t1})$ and pressure anisotropy stress $-\alpha\tau_M$. Assuming $v_n < 0$ (see Fig. 1), the positive and negative signs in equations (3) and (1) refer to $B_n < 0$ and $B_n > 0$, respectively.

Equation (1) forms the basis for our test of the hypothesis that the plasma inside the magnetopause received its high velocity from the magnetic slingshot. We apply it across the first magnetopause crossing, using only data points immediately adjacent to the current sheet. The number density, although it had a substantial maximum within the sheet, had nearly identical values ($\sim 10 \text{ cm}^{-3}$) on the two sides. Equation (2) then indicates that α also should have been the same. For $\rho_2 = \rho_1$ equation (1) predicts that the tangential velocity difference vector should be parallel ($B_n < 0$) or antiparallel ($B_n > 0$) to the magnetic difference vector, and that its magnitude should equal the modified Alfvén speed based on the magnetic difference field. Figure 3 shows the behaviour of $\mathbf{v}$ and $\mathbf{B}$ throughout the first magnetopause crossing. The two difference vectors may be imagined as extending from points ① to points ② on the left in Fig. 3. They are seen to be nearly parallel, forming an angle of

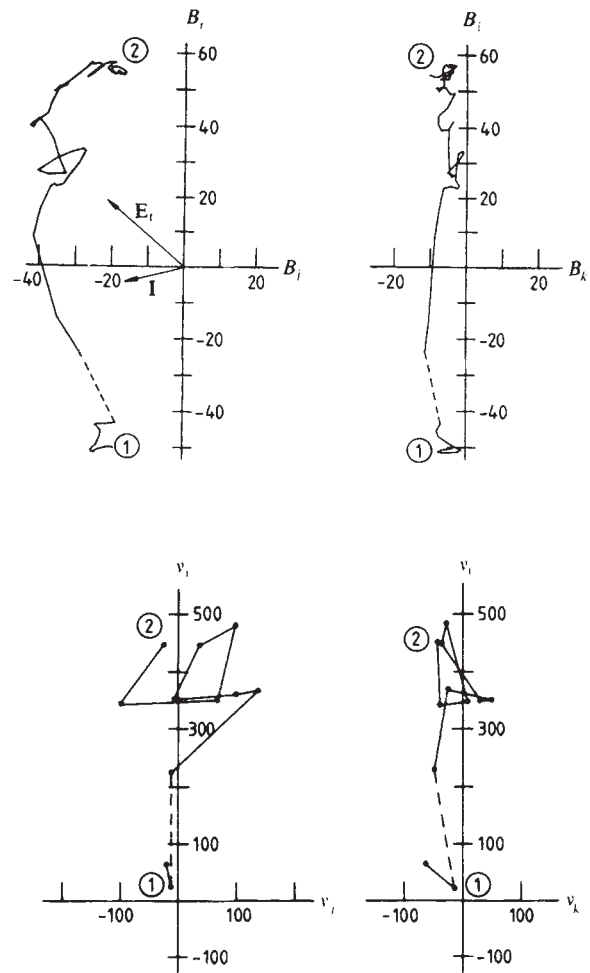


Fig. 3 Polar plots of the magnetic field $\mathbf{B}$ (nT) and plasma flow velocity $\mathbf{v}$ (km s^{-1}) for the first magnetopause crossing of Fig. 2 in the principal axes coordinate system obtained from minimum variance analysis. The k axis is in the direction of minimum variance of the magnetic field and is interpreted as the outward-directed normal to the magnetopause. The (i, j) -plane is tangential to the magnetopause, with the i and j axes approximately due north and west, respectively. Direction cosines with respect to the solar ecliptic x, y, z axes are $(-0.59; -0.37; 0.72)$, $(-0.12; -0.84; -0.53)$ and $(0.80; -0.40; 0.45)$ for the i, j, k axes, respectively. The points marked 1 and 2 refer to the magnetosheath and magnetosphere sides of the magnetopause. Magnetic field and plasma data points are separated by 4 and 12 s, respectively. Data gaps are shown as dashed line segments. Also shown are vectors representing the magnetopause current $\mathbf{I}$, and the electric field $\mathbf{E}_t$.

only 12° . Given the uncertainties associated with temporal variations and with the plasma measurements, this value is not significantly different from zero. The fact that the vectors are parallel rather than antiparallel indicates $B_n < 0$, as expected for a crossing in the Northern Hemisphere (see Fig. 1).

The magnitude of the measured tangential velocity difference is 427 km s^{-1} whereas for $\alpha = 0$, a number density of 10 cm^{-3} , and a 5% abundance of α particles in the proton plasma, the Alfvén speed based on the difference magnetic field is 580 km s^{-1} . We conclude that the difference between these two numbers is not significant. First, gain degradation of the plasma detectors may have led to a systematic underestimation of the density of up to 20% and therefore to an overestimation of the difference Alfvén speed of up to 10%. More importantly, the limited angular resolution of the measurements will lead to an uncertainty (normally an underestimate) of the flow velocity of up to 30% if the flow is inclined $\sim 45^\circ$ with respect to the ecliptic, as in the present case¹¹. Finally, the presence in the magnetopause region of α particles beyond the assumed level and/or of small numbers of heavier ions of ionospheric origin may reduce the difference Alfvén speed further.

Figure 2 shows that high velocity plasma is present, not only in the boundary layer but throughout most of the magnetopause itself, both for the main crossing and for the partial ones. As the magnetopause thickness was large compared with an ion gyro radius, we may compare all of the velocity measurements with equation (1) as follows: We suppress the subscript 2 in that equation and let the corresponding quantities represent conditions at an arbitrary point. Subscript 1 will represent average magnetosheath conditions. Equation (1) then predicts a linear relationship, with slope

$$m = [(1 - \alpha_1)/\mu_0 \rho_1]^{1/2} \quad (5)$$

between $(\rho_1/\rho) \mathbf{B}_i$ and $\mathbf{v}_i$. As Fig. 3 shows, the main dynamical effect is associated with the 'i' components of these two vectors. A scatter plot of $\mathbf{v}_i$ against $(\rho_1/\rho) \mathbf{B}_i$ is shown in Fig. 4. Linear regression analysis of these data yields a correlation coefficient of 0.90 and a slope of 4.4 ± 0.3 ($\text{km s}^{-1} \text{ nT}^{-1}$). On the other hand, the slope obtained from equation (5) with $\alpha_1 = 0$ and an average magnetosheath particle density ρ_1 (see Fig. 2) of 12.8 cm^{-3} , is $m = 5.6$ ($\text{km s}^{-1} \text{ nT}^{-1}$). In view of the uncertainties discussed previously, the difference between the two slopes is not significant. We conclude that the entire set of data is compatible with the slingshot acceleration hypothesis.

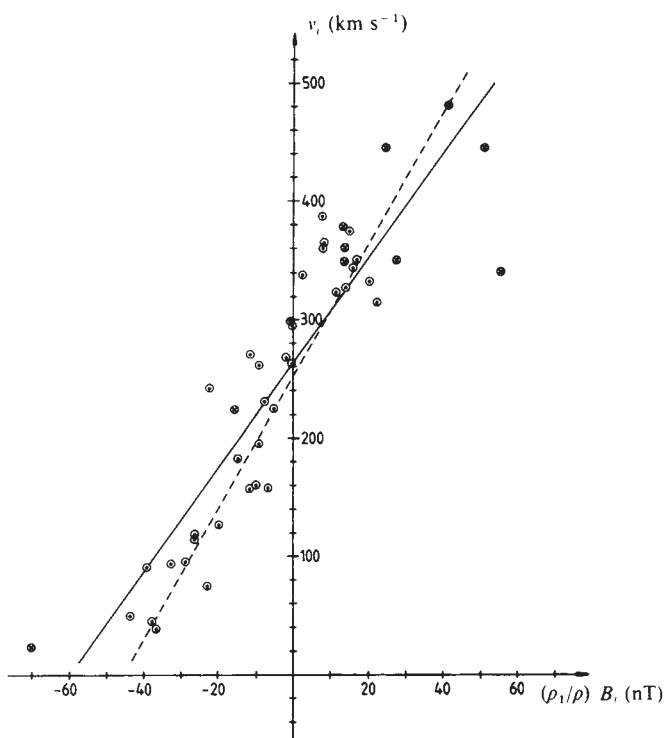


Fig. 4 Scatterplot of v_i against $(\rho_1/\rho) B_i$ for the magnetopause encounters of Fig. 2. ⊙, 00.42.20–00.44.20 UT interval; ○, 00.45.56–00.52.56 UT interval. v_i and B_i are the components of $\mathbf{v}_i$ and $\mathbf{B}_i$ along the i axis in Fig. 3. The index 1 refers to average magnetosheath conditions ($\rho_1 = 12.8 \text{ cm}^{-3}$). Index-less symbols refer to measurements in the intervals above. The solid line is a fit to the data using a linear regression analysis, the dashed line marks the slope predicted by equation (5) with $\alpha_1 = 0$ and $\rho_1 = 12.8 \text{ cm}^{-3}$.

As already mentioned, the slingshot acceleration process requires the presence of a magnetic field component B_n perpendicular to the magnetopause. Furthermore, the direction change of the tangential plasma velocity in the event discussed here corresponds to a negative value of B_n . To check this prediction, we have calculated the normal vector $\mathbf{n}$ and the average normal magnetic field component B_n directly from the magnetic data during the first crossing by the minimum variance technique^{16,17}. The results depend on the precise time interval chosen, with B_n ranging from -3 to -9 nT . The normal coordinate system used in Fig. 3, and the subsequent calculations of v_n

and E_i are based on the results from a time segment with $B_n = (-5.4 \pm 2.9) \text{ nT}$. This value is close to the average and has the smallest absolute error. With $B_n = -5.4 \text{ nT}$, an average density $N_p = 15 \text{ cm}^{-3}$ and $\alpha = 0$, we find (equation (1)) $v_n = -28 \text{ km s}^{-1}$ for the first magnetopause crossing. Because of the inward motion, at 10 km s^{-1} , of the magnetopause relative to the satellite, the predicted normal plasma velocity is -38 km s^{-1} . As shown in Fig. 3, the measured velocity component v_k is in fact negative on the average, albeit with large fluctuations. Figure 3 illustrates the difficulty of establishing the presence or absence of plasma flow across the magnetopause by direct examination of v_k .

In a time-dependent one-dimensional magnetopause, the tangential electric field E_t , measured in the frame moving with the magnetopause, should remain constant during a crossing. This field may be obtained from the simplified Ohm's law¹⁸:

$$\mathbf{E}_t = -\mathbf{v}_n \times \mathbf{B}_t - \mathbf{v}_t \times \mathbf{B}_n \quad (6)$$

The largest correction term to equation (6) would be the Hall term¹⁸. However, for a thick layer this term contributes very little. Using the values of B_n and v_n derived previously, we find $E_t = 1.8 \pm 0.5 \text{ mV m}^{-1}$ during the first crossing. The electric field vector forms an angle of $56^\circ \pm 10^\circ$ with the total current $\mathbf{I}$, which was deduced from the jump in $\mathbf{B}_t$ (see Fig. 3). This result supports Cowley's¹⁹ conclusion, arrived at from theoretical considerations, that the two vectors need not be parallel during reconnection.

The dissipation rate $\mathbf{E} \cdot \mathbf{I}$, with $I = 0.073 \text{ A m}^{-1}$, is found to be $78 \pm 24 \text{ W km}^{-2}$. Both the electric field and the dissipation rate are comparable to those obtained from the d.c. electric field experiment²⁰ on ISEE 1 for another crossing²¹. Unfortunately, that experiment was in an unfavourable mode during the present event, so that no comparison is possible.

Conclusions

Our observations provide the first evidence for the occurrence of the plasma acceleration at the magnetopause intrinsic to the reconnection process. So far two more examples of this kind have been found in the ISEE data. However, in most cases with favourable magnetic field orientation no plasma acceleration is observed. This means either that reconnection occurs rarely and in conditions which are not yet understood, or that the process is small-scale and non-stationary, and therefore has a low probability of being observed locally.

The Max Planck Institut portions of this work were supported by the Bundesministerium für Forschung und Technologie under grant RV14-B6/74. Los Alamos portions were done under the auspices of the US Department of Energy with NASA support under S-50864A. Work at UCLA was supported under NASA contract NAS-5-20064.

Received 21 August; accepted 17 September 1979.

- Dungey, J. W. *Phys. Rev. Lett.* **6**, 47–48 (1961).
- Petschek, H. E. *NASA Spec. Publ.* SP-50, 425–439 (1964).
- Levy, R. H., Petschek, H. E. & Siscoe, G. L. *Am. Inst. Aeronaut. Astronaut. J.* **2**, 2065–2076 (1964).
- Hones, E. W., Jr., Bame, S. J. & Asbridge, J. R. *J. geophys. Res.* **81**, 227–234 (1976).
- Frank, L. A., Ackerson, K. L. & Lepping, R. P. *J. geophys. Res.* **81**, 5859–5881 (1976).
- Sonnerup, B. U. Ö. *Solar System Plasma Physics* Vol. III (eds Lanzerotti, L. T., Kennel, C. F. & Parker, E. N.) 45–108 (North-Holland, Amsterdam, 1979).
- Ogilvie, K. W., von Rosenvinge, T. & Durney, A. C. *Science* **198**, 131–138 (1977).
- Yang, C.-K. & Sonnerup, B. U. Ö. *J. geophys. Res.* **82**, 699–703 (1977).
- Heikkilä, W. *J. Geophys. Res. Lett.* **2**, 154–157 (1975).
- Bame, S. J. *et al.* *IEE Trans. Geosci. Electron.* **GE-16**, 216–220 (1978).
- Paschmann, G. *et al.* *Space Sci. Rev.* **22**, 717–737 (1978).
- Russell, C. T. *IEEE Trans. Geosci. Electron.* **GE-16**, 239–242 (1978).
- Eastman, T. E. & Hones, E. W. Jr. *J. geophys. Res.* **84**, 2019–2028 (1979).
- Spreiter, J. R., Alksne, A. Y. & Summers, A. L. *Physics of the Magnetosphere* (eds Carovillano, R. L., McClay, J. F. & Radoski, H. R.) 301–375 (Reidel, Dordrecht, 1968).
- Hudson, P. D. *Planet. Space Sci.* **18**, 1611–1622 (1970); **19**, 1693–1699 (1971); **21**, 475–483 (1973).
- Sonnerup, B. U. Ö. & Cahill, L. J. *J. geophys. Res.* **72**, 171–183 (1967).
- Sonnerup, B. U. Ö. *J. geophys. Res.* **76**, 6717–6735 (1971).
- Rossi, B. & Olbert, S. *Introduction to the Physics of Space*, 350, 355 (McGraw-Hill, New York, 1970).
- Cowley, S. W. H. *J. geophys. Res.* **81**, 3455–3458 (1976).
- Mozer, F. S. *et al.* *IEEE Trans. Geosci. Electron.* **GE-16**, 258–260 (1978).
- Mozer, F. S. *et al.* *Geophys. Res. Lett.* **6**, 305–308 (1979).
- Haerendel, G., Paschmann, G., Skopke, N., Rosenbauer, H. & Hedgecock, P. C. *J. geophys. Res.* **83**, 3195–3216 (1978).

Rare earth element mobility associated with uranium mineralisation

Scott M. McLennan & S. R. Taylor

Research School of Earth Sciences, The Australian National University, Canberra A.C.T. 2600, Australia

Extreme rare earth element mobility has been discovered, associated with uranium mineralisation in metasedimentary rocks of the Lower Proterozoic Pine Creek Geosyncline, Australia. The rare earth element patterns and other geochemical evidence point to movement of the rare earth elements and uranium by carbonate complexes in a low temperature, oxidising and alkaline solution.

MOBILITY of rare earth elements (REEs) in rocks is of interest as these elements are often used as petrogenetic indicators. The REEs seem to be immobile in most metamorphic conditions¹⁻³. Some light rare earth element (LREE, La-Sm) mobility has been detected during hydrothermal alteration (submarine weathering), spilitisation and lower greenschist metamorphism associated with hydrothermal activity⁴⁻⁶. Collerson and Fryer⁷ suggested heavy rare earth element (HREE, Gd-Lu) mobility due to carbonate complexing associated with granulite metamorphism and granite production in Archean terrains. Several studies have related HREE mobility to complexing (mainly carbonate complexing) in hydrothermal conditions⁸⁻¹³. The present study examines the relationship between REE and uranium mineralisation in the Cahill Formation¹⁴ of the Pine Creek Geosyncline, Northern Territory, Australia.

Geological setting

The following summary of the geology and geochemistry of the Pine Creek Geosyncline is based on information given at the International Uranium Symposium on the Pine Creek Geosyncline, Sydney, 3-8 June, 1979.

The Pine Creek Geosyncline (Fig. 1) comprises up to 14 km of mainly Lower Proterozoic metasedimentary rocks (clastics and carbonates)¹⁵. These rocks were deposited between age limits of 2,400 and 1,870 Myr (ref. 16).

Most of the important uranium deposits in the eastern part of the Pine Creek Geosyncline are contained within the Cahill Formation (Fig. 1). This unit consists primarily of quartzofeldspathic and micaceous metasedimentary rocks. A lower member (which contains most uranium-deposits) contains abundant carbonate and carbonaceous metasedimentary rocks¹⁵. The exact age of the Cahill Formation is in some doubt. Most workers^{14,15,17} suggest it is of Lower Proterozoic age. Consideration of metamorphic assemblages¹⁸, however, indicates that these rocks may have been buried deeper than can be explained by the known Lower Proterozoic sedimentary thickness and that an age as old as Archean cannot be excluded.

Uranium mineralisation, for the most part, is strata-bound and occurs in breccia matrix and vugs. In most samples, chlorite is ubiquitous, primarily as an alteration product¹⁹. The favoured model is that uranium deposition occurred from a low temperature fluid within collapse structures (caves, cavities) of an impure carbonate stratum which has undergone extensive solution and development of karst terrain²⁰. Alternative models have been described by Binns *et al.*²¹ and Crick and Muir²². Ore deposition probably occurred between about 1,800 and 1,700 Myr (refs 20, 23).

Most samples analysed in this study probably represent the insoluble residue (plus alteration products, ore minerals and so

on) after carbonate had been removed. Samples include fine-grained metasedimentary schists with a wide range of uranium concentrations (1.4-48,230 p.p.m.). The least altered sample (PCG132) is a muscovite-biotite-quartz schist ($\text{SiO}_2 = 77.44\%$). Modal mineralogy of the altered samples is rather variable although chlorite (primarily as an alteration product) and quartz are always dominant. Muscovite, graphite and haematite are also occasionally present, in most samples. One sample of massive, impure uraninite (uraninite + fourmarierite + secondary U and Pb minerals) was also analysed.

REE, Th and U data

Four samples were analysed for REE (excluding Pr + Ho) by an ion-exchange/X-ray fluorescence technique and the remainder by spark source mass spectrometry. Details of techniques have been reported previously^{24,25}. Uranium and thorium concentrations on samples PCG52, PCG57 and PCG148 and U concentration on sample 75080180 were made available by John Ferguson of the Bureau of Mineral Resources, Canberra, Australia. Results of REE, U and Th analyses are given in Table 1.

As most of these rocks were originally sedimentary, it is convenient to compare REE data to typical sedimentary rocks. Figures 2 and 3 plot the REE data normalised to Post-Archean Average Australian Sedimentary rock (PAAS)²⁶.

Sample PCG132 has a U concentration (1.4 p.p.m.) and Th/U ratio (4.7) well within the range of typical sedimentary rocks and may be considered a normal, unmineralised sample. The REE pattern of this sample (Fig. 2) is nearly parallel to PAAS, although lower in total abundances by a factor of about 0.5-0.7. Minor HREE depletion is apparent and there is no anomalous Eu behaviour relative to PAAS. The normal distribution of REE in clastic sedimentary rocks is extremely uniform and similar to PAAS²⁶. It is therefore likely that PCG132 is fairly typical of unaltered samples in the Cahill Formation.

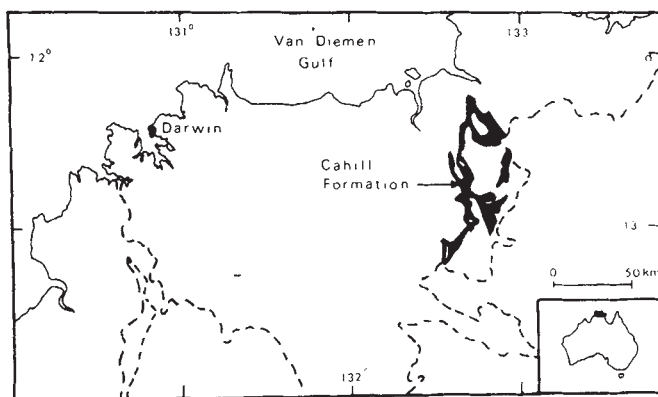


Fig. 1 Location map of the Pine Creek Geosyncline, N.T., Australia. The approximate limits of the geosyncline are marked by dashed lines and the Cahill Formation is shown in black.

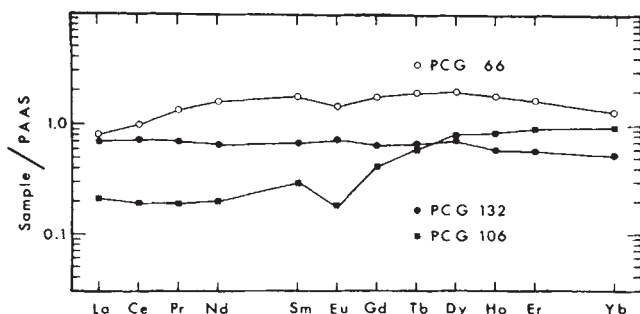


Fig. 2 REE patterns, normalised to post-Archean average Australian shales (PAAS)^{26,36}, for samples with low and intermediate U content.

Samples with intermediate U content (PCG66, PCG106) show REE patterns (Fig. 2) which differ substantially from PAAS but there is no consistent trend. PCG106 is notable for LREE depletion and a negative Eu anomaly. Samples enriched in U (PCG57, PCG148, PCG52, 75080180) show consistent REE trends (Fig. 3). They are depleted in LREE and enriched in HREE. These samples show a PAAS-normalised maximum in the vicinity of Tb-Dy.

The impure uraninite sample (75080260) has a REE pattern which parallels the U-rich samples (Fig. 3). Uraninite alone, however, cannot account for the REE concentrations of the other samples. Mixing calculations between PCG132 and 75080260 result in about an order of magnitude excess in total REE (Σ REE) for most samples with U concentrations comparable to those resulting from the mixing proportions. Also, LREE depletion cannot be achieved by such mixing.

For all metasedimentary rock samples, there is an inverse correlation between U content and La:Yb ratio (Fig. 4). Samples with $U \geq 1,900$ p.p.m. show a correlation between U content and Σ REE (see Fig. 3). Another interesting feature of these rocks is the complete lack of coherence between U and Th, as indicated by the strong inverse correlation between U content and Th:U ratio (Fig. 5). This is in strong contrast to the general behaviour of Th and U in most igneous and sedimentary rocks²⁷.

The remarkable correlation between REE and U content, in these samples, has not been previously observed. The REE patterns in the U-rich samples (Fig. 3) are unlike any terrestrial³ or extraterrestrial²⁸ rock. Common rock forming minerals such as garnet and zircon do display HREE enrichment³, but they are not present in significant proportions and could not explain these REE patterns. No REE minerals have been identified in these samples which could explain such patterns and it is possible that much of the REE content was associated with the original clay minerals and alteration products (now chlorite, biotite, muscovite, and so on). Significant adsorption of REE on clay minerals has been documented for Quaternary clays of Norway²⁹.

REE mobility

The above observations indicate that massive REE mobility related to U movement, resulting in depletion of LREE and enrichment of HREE, occurs relative to the parent rock. The

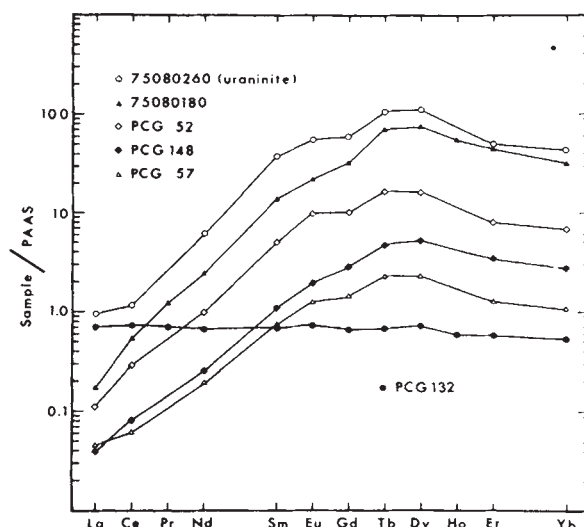


Fig. 3 REE patterns, normalised to PAAS, for samples with high U content. Sample PCG132 shown for comparison.

Table 1 Trace element analyses of samples from the Cahill Formation, Pine Creek Geosyncline (p.p.m.)

Sample	PCG52	PCG57	PCG66	75080180	PCG106	PCG132	PCG148	75080260	PAAS§
Th	16	13	20	15	18	6.6	9	—	
U	12,580	1,900	455	48,230	229	1.4	3,720	—	
Th/U	0.0013	0.0068	0.044	0.0003	0.079	4.7	0.0024	—	
La	4.2	1.7	31	5.5	7.9	27	1.5	30	38
Ce	23	4.8	79	43	15	58	6.5	92	80
Pr	—	—	12	11	1.7	6.2	—	—	8.9
Nd	32	6.2	52	77	6.5	21	7.9	200	32
Sm	28	4.2	10	79	1.7	3.9	5.8	214	5.6
Eu	11	1.4	1.6	24	0.20	0.80	2.1	61	1.1
Gd	47	6.6	8.4	153	2.0	3.1	13	277	4.7
Tb	13	1.8	1.5	55	0.47	0.53	3.6	83	0.77
Dy	73	10	8.8	329	3.6	3.2	23	490	4.4
Ho	—	—	1.8	55	0.84	0.58	—	—	1.0
Er	23	3.6	4.8	128	2.7	1.7	10	144	2.9
Yb	19	2.9	3.7	89	2.6	1.5	7.9	127	2.8
Σ REE†	294.8	47.2	215.8	1075.5	46.0	128.0	89.3	1853	
Eu/Eu*‡	0.94	0.82	0.54	0.68	0.34	0.71	0.75	0.78	
La/Yb	0.22	0.59	8.4	0.062	3.0	18.0	0.19	0.24	

Samples used—PCG52 (graphite-chlorite-quartz schist); PCG57 (chlorite-quartz schist); PCG66 (chlorite-muscovite-quartz schist); 75080180 (quartz-mica-chlorite-haematite schist); PCG106 (chlorite-muscovite-quartz schist); PCG132 (muscovite-biotite-quartz schist); PCG148 (muscovite-chlorite-quartz schist); 75080260 (impure uraninite) and PAAS.

† Estimated.

‡ Eu* is a theoretical value for no chondrite-normalised Eu-anomaly.

§ PAAS—Post-Archean average Australian sedimentary rock; values from Nance and Taylor²⁶.

LREE depletion shows that REE were leached from the sedimentary rocks themselves. The HREE were selectively deposited with the U or selectively incorporated into the ore depositing solution. A HREE-depleted parent lithology has not been identified but slight HREE depletion was apparent in PCG132, the supposedly unaffected sample. If large volumes of parent rock were involved, a notable HREE depletion may not occur.

The nature of the solution which carried the REE and U can be determined from several lines of evidence. The lack of Th-U coherence (Fig. 5) strongly suggests that U was primarily transported in solution as U^{6+} . Complexes of uranous ions with chlorides, sulphates, phosphates or fluorides are thus unlikely transporting mechanisms^{27,30}. Transport of U as uranyl ions (UO_2^{2+}), carbonate complexes, uranyl fluoride complexes or uranyl phosphate complexes are all possible³⁰. Although several secondary uranium sulphate and uranium phosphate minerals have been reported from this area, lack of correlation between U and S, F or P³² suggests that sulphate, fluoride and phosphate complexes were unimportant.

REE mobility, under hydrothermal conditions, is also best accomplished by soluble complexes. The most common forms of complexing are sulphate, fluoride and carbonate⁸. Sulphate complexing generally results in major hydrothermal sulphate deposits⁸. Pseudomorphs after sulphate minerals have been recognised in this region but are thought to be of evaporitic origin²². The lack of correlation between REE (La, Ce, Y) and F or S³² indicates that these complexes were probably unimportant.

It seems most likely that U and REE were transported together in a hydrothermal fluid as soluble carbonate complexes. Typical forms would be $[UO_2(CO_3)_3]^{4-}$ and $[REE(CO_3)_3]^{3-}$. Transport of U as simple uranyl ions may also have occurred but is not considered important since this species only predominates at relatively low pH³⁰. The shape of the REE patterns for the U-rich samples is easily explained by such a mechanism since complexes of the HREE tend to be more soluble than LREE⁸.

Strong supporting evidence for this mechanism is available from fluid inclusion studies³³. Inclusions in quartz, thought to be associated with the main stage of U-mineralisation (Type II inclusions of Ypma and Fuzikawa³³) contain CO_2 as a major phase (along with H_2O and CH_4). The temperatures of such fluids are difficult to determine but very low (meteoric?) values have been suggested²⁰. REE and U carbonate complexes of the type suggested here would be best formed under alkaline ($pH \geq 7.5$) and oxidising ($E_H \geq 0.2$ V) conditions with temperatures below about 100 °C (refs 8, 30).

The conditions in which deposition occurred are more speculative. Lowering of pH, E_H and carbonate activity are all efficient mechanisms⁸. The activity of CO_2 would be effectively

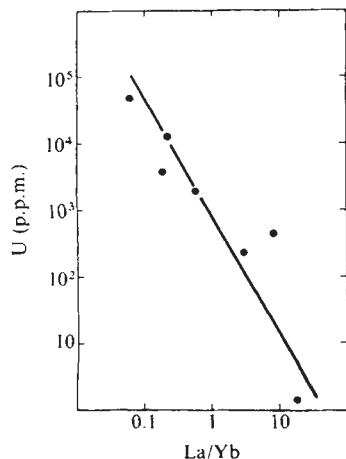


Fig. 4 Plot of U content against La/Yb for whole rock samples.

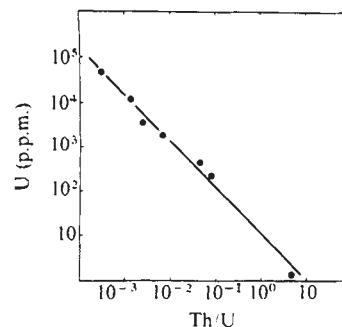


Fig. 5 Plot of U content against Th/U for whole rock samples.

decreased by the lowering of total pressure on entrance of the ore-bearing solutions into cavities⁸. Such a mechanism would be in accordance with the karst environment suggested for these ores^{18,19}.

Most other studies which have appealed to carbonate complexes for mobilisation of REE have dealt with HREE depletion^{7,13}. These studies, however, considered fairly high temperature regimes. In a study of the Precambrian Sokoman iron-formation, Fryer¹² suggested REE mobility by carbonate complexes during diagenesis to explain HREE enrichment. It seems that HREE are preferentially retained in hydrothermal solutions and only removed in late stage and/or low temperature deposits. This is consistent with present understanding of chemistry of REE carbonate complexes^{8,9}.

A final point which deserves comment is the behaviour of Eu in these rocks. If soluble carbonate complexes are controlling REE distribution in these rocks, the Eu^{2+} would be expected to behave very differently than REE^{3+} . This should result in anomalous Eu behaviour with increasing U-content; this clearly does not occur (Fig. 3). It seems unlikely that a second major complexing agent was present (such as halogens and phosphates) allowing transport of Eu^{2+} (see above discussion). Equally unlikely is the possibility that Eu^{2+} was oxidised to Eu^{3+} by the ore-forming fluid since other evidence²⁰ suggests that the fluid varied only from moderately reducing to moderately oxidising. An intriguing possibility is that the source material contained only Eu^{3+} . If the source were sedimentary it may indicate that Eu^{2+} can be oxidised to Eu^{3+} during sedimentary processes and still preserve a memory of Eu-anomalies inherited from the igneous precursors^{26,34-36}.

These results have important implications for a wide range of geochemical problems. It is now quite clear that the REEs can be transported as soluble carbonate complexes in fluid phases. REE studies on rocks which have been significantly affected by fluid migration and alteration must take this factor into account.

We thank J. Ferguson for samples and several U and Th analyses, Brian J. Fryer for four REE analyses and P. E. Oswald-Sealy, J. M. G. Shelley, H. N. C. Cutten and W. Sinclair for technical assistance.

Received 5 July; accepted 7 September 1979.

- Green T. H., Brunfelt, A. O. & Heier, K. S. *Earth planet. Sci. Lett.* **7**, 93-98 (1969); *Geochim. cosmochim. Acta* **36**, 241-257 (1972).
- Cullers, R. L., Yeh, L.-T., Chadhuri, S. & Guidotti, C. V. *Geochim. cosmochim. Acta* **38**, 389-400 (1974).
- Herrmann, A. G. *Handbook of Geochemistry* Vol. 39 (ed. Wedepohl, K. H.), 57-71 (Springer, Berlin, 1970).
- Hellman, P. L. & Henderson, P. *Nature* **267**, 38-40 (1977).
- Floyd, P. A. *Nature* **269**, 134-137 (1977).
- Ludden, J. N. & Thompson, G. *Nature* **274**, 147-149 (1978).
- Collerson, K. D. and Fryer, B. J. *Contr. Miner. Petrol.* **67**, 151-167 (1978).
- Kosterin, A. V. *Geochemistry*, 381-387 (1959).
- Beus, A. A. *Geochemistry*, 388-396 (1958).
- Minyev, D. A. *Geochemistry*, 1129-1149 (1963).
- Tugarinov, A. I. & Naumov, V. B. *Geochem. Int.* **9**, 161-167 (1972).
- Fryer, B. J. *Can. J. Earth Sci.* **14**, 1598-1610 (1977).
- Kerrick, R. & Fryer, B. J. *Can. J. Earth Sci.* **16**, 440-458 (1979).
- Needham, R. S. & Stuart-Smith, P. G. *Bur. miner. Res. J. Aust. Geol. Geophys.* **1**, 321-333 (1976).
- Needham, R. S., Crick, I. H. & Stuart-Smith, P. G. *Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).

16. Page, R. W., Compston, W. & Needham, R. S. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
17. Stuart-Smith, P. G., Wills, K., Crick, I. H. & Needham, R. S. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
18. Ferguson, J. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
19. Ewers, G. R. & Ferguson, J. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
20. Ferguson, J., Ewers, G. R. & Donnelly, T. H. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
21. Binns, R. A., McAndrew, J. & Sun, S.-S. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
22. Crick, I. H. & Muir, M. D. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
23. Hills, J. H. & Richards, J. R. *Miner. Deposita* **11**, 133–154 (1976).
24. Fryer, B. J. *Geochim. cosmochim. Acta* **41**, 361–367 (1977).
25. Taylor, S. R. & Gorton, M. P. *Geochim. cosmochim. Acta* **41**, 1375–1380 (1977).
26. Nance, W. B. & Taylor, S. R. *Geochim. cosmochim. Acta* **40**, 1539–1551 (1976).
27. Adams, J. S., Osmond, J. K. & Rogers, J. J. W. *Phys. Chem. Earth* **3**, 298–348 (1959).
28. Taylor, S. R. *Lunar Science: a Post-Apollo View* (Pergamon, Oxford, 1975).
29. Roaldset, E. *Lithos* **6**, 349–372 (1973).
30. Langmuir, D. *Geochim. cosmochim. Acta* **42**, 547–569 (1978).
31. Snelling, A. A. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
32. Ferguson, J. & Winer, P. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
33. Ypma, P. J. M. & Fuzikawa, K. *Int. Uranium Symp. Pine Creek Geosyncline*, (IAEA, Vienna, in the press).
34. Nance, W. B. & Taylor, S. R. *Geochim. cosmochim. Acta* **41**, 225–231 (1977).
35. McLennan, S. M., Fryer, B. J. & Young, G. M. *Geochim. cosmochim. Acta* **43**, 375–388 (1979).
36. Taylor, S. R. *The Earth: Its Origin, Structure and Evolution* (ed. M. W. McElhinny) 353–376 (Academic, New York, 1979).

Vesicularity and CO₂ in mid-ocean ridge basalt

James G. Moore

US Geological Survey, Menlo Park, California 94025

Vesicles and included CO₂ are enriched in deep-sea basalts that are also enriched in light rare earth and incompatible elements. This enrichment probably results from a unique deep mantle origin of such melts but may have been modified by CO₂ bubbles rising in shallow magma chambers.

THE primary control on the vesicularity of subaqueously erupted basalt is the depth (and hydrostatic pressure) of eruption^{1,2}. Shallower eruption produces more (and larger) vesicles not only because exsolved volatiles expand with lower pressure, but also because exsolution of volatiles dissolved in the melt is more complete. However, basalt composition also affects the vesicularity of submarine basalt. Alkaline basalt seems to be more vesicular (at the same depth) than tholeiitic basalt³, although fresh submarine alkaline basalt sample suites are rare and data limited. Fresh tholeiitic basalt from the same general area on a mid-ocean ridge such as the Mid-Atlantic Ridge at 37°N shows a variation in vesicularity that can be related to major element variations, such as K₂O (ref. 4).

In addition to these vesicularity controls, important variations have been noted between tholeiitic basalts from different mid-ocean ridges which seem to be independent of depth or major element chemistry⁵. These variations are of such magnitude that individual suites from mid-ocean ridges can be characterised by their vesicularity.

Analyses of the gas contained in vesicles from four localities on spreading ridges indicates that >95% of the remaining gas is CO₂ (ref. 6). Hence the vesicularity of submarine basalts (except for those that are shallower than about 1 km) is a measure of the CO₂ content of the lava (other than that dissolved in the glass).

In this study, vesicularity has been determined by point counting standard thin sections of the outermost 1–1.5 cm of pillows and slabs, most of which include at least some sideromelane glass. More than 1,000 points were counted, and the vesicularity is reported in volume per cent of vesicles in glass (or microcrystalline material) excluding phenocrysts and microphenocrysts. More than 200 samples have been analysed from 13 ridge segments in the Atlantic and Pacific oceans (Table 1).

Results

The measurements of the volume per cent vesicles at any locality vary widely, particularly in rocks with a low vesicle content, but show a rough log-normal distribution (Fig. 1). For lava suites with ~10% vesicles, the variation is about one-half order of

magnitude, but for suites of low vesicularity, the variation is as much as 1.5 orders (Fig. 1). This is partly a measurement problem, as only one point out of 1,000 would fall on a vesicle for a rock giving 0.1% vesicles. In practice, counting was continued on the very vesicle-poor rocks until at least one point fell on a vesicle. Because of the log-normal distribution, the logarithmic mean is used as a measure of the vesicularity of a given suite (Table 1). Among lavas collected (and presumably erupted) at about the same depth, the logarithmic mean vesicularity between sample suites varies by an order of magnitude (Figs 1, 2 and Table 1). The extremes in the variation are clearly real and not a result of measurement problems (compare Figs 1 and 2).

The effect of depth of eruption on vesicularity can be partly evaluated by comparing the vesicularity averages with the trend line established independently from samples collected at known depth on the east rift zone of Kilauea volcano and the south rift zone of Mauna Loa volcano, Hawaii. Thirty-five samples were analysed from 16 dredge hauls through a depth range of 490–5,190 m. The least-squares fit of the data on a log plot is shown in Fig. 2. The straight line on the log plot is defined by $\log V = 6.25 - 1.88 \log D$ where V is the volume per cent vesicles and D is the depth in metres. Hence V is very nearly proportional to D^{-2} . Such a relationship is consistent with the increase of V towards shallower depth resulting from both the Boyle's law increase in vesicle size (due to reduction of pressure) and

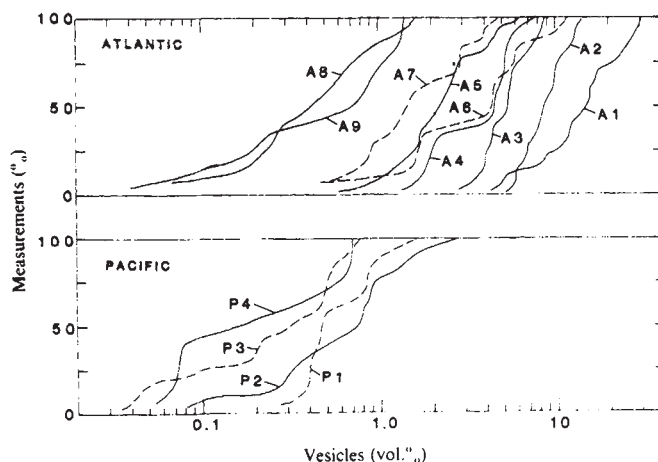


Fig. 1 Cumulative curves of volume per cent vesicles for the outermost 1–1.5 cm of basalt lava flows from 13 ocean ridge segments (see Table 1).

exsolution of an additional gas phase whose mass is proportional to the reduction in pressure⁶. Therefore, the logarithmic mean vesicularity of sample suites has been projected to a common depth of 3 km parallel to a line with a slope of $V = 1/D^2$ (Table 1, Fig. 2).

The logarithmic mean vesicularity at 3 km ranges from 0.17 to 2.9%, and the Atlantic suites are consistently higher (>0.55%) than those of the Pacific. The adjusted average vesicularity for the Reykjanes Ridge suites (A1, A2, A3) is least reliable because they are shallow and have been extrapolated through the greatest depth range.

The presence of the high-vesicle suites in the Atlantic suggests that they may be favoured by the slower spreading which prevails in the Atlantic. A plot of vesicularity adjusted to 3 km relative to spreading rate (Fig. 3) indicates a rough inverse correlation. Pacific suites range from 0.17 to 0.51% vesicularity and 3 to 8 cm yr⁻¹ half spreading rate. Atlantic suites range from 0.56 to 2.9% vesicularity and all spread at about 1 cm yr⁻¹.

Schilling⁷ has noted that spreading ridge basalts enriched in the volatile elements Cl, F and Br are also enriched in other incompatible elements, the light rare earth elements (LREE), and show high ⁸⁷Sr/⁸⁶Sr ratios. The chondrite normalised ratio La/Sm, which is a measure of LREE enrichment, has been previously determined in rocks from 11 of the 13 segments (see references of Table 1). Samples analysed for rare earth elements are partly the same samples analysed for vesicles, and partly samples which were obtained from localities close to those analysed for vesicles. The LREE enrichment factor (La/Sm)EF shows a moderately good correlation with adjusted vesicularity (Fig. 3). The so-called 'normal ridge basalts' with (La/Sm)EF of about 0.6 show log-mean vesicularities adjusted to 3 km depth <0.7%, and areas enriched in the halogens with higher (La/Sm)EF range in vesicularity up to 2.9% (Fig. 3).

The variation in adjusted vesicularity can be regarded as a measure of the CO₂ content of the rock in excess of saturation as CO₂ is the predominant gas in vesicles. The vesicle CO₂ in the rocks ranges from about 70 p.p.m. for the Galapagos samples (P3) to 1,200 p.p.m. for samples collected from 45° N on the Mid-Atlantic Ridge (A4) assuming a rigid temperature of the basalt glass of 1,000 °C.

The size of the vesicles ranges widely in a single thin section, but vesicles in the matrix glass (excluding glass inclusions in

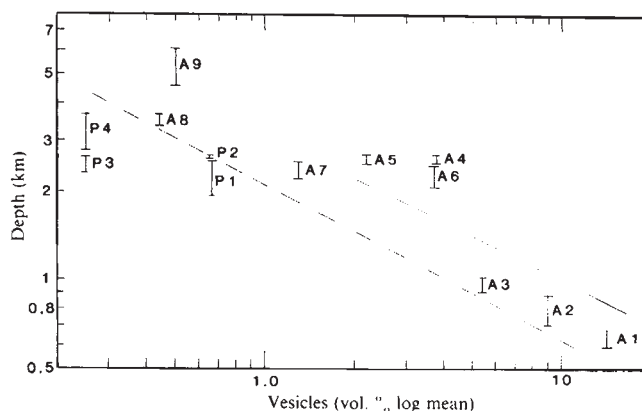


Fig. 2 Logarithmic mean vesicularity relative to depth range for 13 suites of mid-ocean ridge basalt samples. Dashed line is least-squares logarithmic trend line for Hawaiian rift zone samples. All sample suites have been projected parallel to solid line to estimate mean vesicularity at 3 km depth (see Table 1).

phenocrysts) show a distinct minimum diameter of 3–4 µm. This minimum diameter may be controlled by the surface tension of the melt. The logarithmic median diameter decreases with depth. It is 40–45 µm at 2.5 km depth and 20–25 µm at 5 km depth, but even at this depth, some vesicles attain 100–200 µm in diameter. Hence, a small population of small vesicles must exist in the upper part of magma chambers a few kilometres beneath oceanic spreading ridges.

Vesicularity controls

The control of the vesicularity of submarine lava is complex because of the transient nature of gas bubbles in melt and the many processes that can operate to enrich, remove, or disperse vesicles. Bubbles tend to rise and concentrate or escape more rapidly than heavy phenocrysts of the same size which would sink because of their greater density contrast with the melt. The processes that control vesicularity may be divided into three general groups depending on their location: (1) during and after eruption on the ocean floor, (2) within magma chambers before

Table 1 Vesicularity of submarine spreading ridge basalts

Symbol	Area	Lat	Long	Depth (m)	No. of measurements	Vesicularity (vol. %)				Vesicle CO ₂ (p.p.m.)	Refs
						Mean	log median	log mean	log mean/3 km		
A1	Reykjanes Ridge	61.7–62.8° N	25.2–26.8° W	923–1040	17	16.7	15.0	14.2	0.64	270	2, 8
A2	Reykjanes Ridge	60.0–62.3° N	25.8–29.5° W	710–900	15	9.3	8.5	9.0	0.62	260	2, 8
A3	Reykjanes Ridge	59.9–60.5° N	29.2–29.7° W	600–687	13	5.6	5.4	5.4	0.56	230	2, 8
A4	Mid-Atlantic Ridge	45.2° N	27.9° W	2527–2697	9	4.2	4.5	3.8	2.9	1200	9
A5	Mid-Atlantic Ridge	36.8–36.9° N	33.2–33.3° W	2512–2735	62	2.6	2.4	2.2	1.7	710	4, 10, 11
A6	Mid-Atlantic Ridge	36.7–36.8° N	33.3° W	2100–2500	11	4.9	4.3	3.8	2.3	960	10 [†]
A7	Mid-Atlantic Ridge	36.4–36.5° N	33.6–33.7° W	2230–2560	17	2.0	1.5	1.3	0.80	330	10 [†]
A8	Mid-Atlantic Ridge	23.0–23.2° N	44.9° W	3392–3685	14	0.59	0.41	0.44	0.60	250	12, 13 [‡]
A9	Cayman Trough	18.0–18.7° N	81.7–81.8° W	4610–6142	13	0.70	0.73	0.50	1.6	670	14 [§]
P1	Juan de Fuca Ridge	46.1–46.9° N	129.3–130.1° W	1950–2550	12	0.99	0.77	0.66	0.33	140	15
P2	East Pacific Rise	20.9° N	109.0–109.1° W	2635–2675	7	0.74	0.48	0.65	0.51	210	5
P3	Galapagos Ridge	0.8° N	86.1–86.2° W	2330–2560	19	0.36	0.32	0.25	0.17	70	16 [¶]
P4	East Pacific Rise	27.7–28.1° S	112.9–113.2° W	2800–3713	5	0.37	0.25	0.25	0.28	120	17

* Calculated from log mean vesicularity at 3 km depth assuming all vesicle gas is CO₂ and rigid temperature is 1,000 °C. Personal communications from: † C. A. Hopson; ‡ F. A. Frey; § J. G. Schilling; ¶ J. B. Corliss.

eruption and within feeding dykes during eruption, and (3) within the source region where magma is generated.

(1) During eruption, the rapid quenching and solidification of pillow basalts on the sea floor is believed to inhibit both the loss of volatiles by bubbles escaping from the lava and the interaction of lava with ocean water. However, recent discoveries on the Galapagos spreading ridge¹⁸ reveal large fluid, non-pillowed lava flows which commonly pond in depressions. Chimneys around the margins of these flows suggest that water trapped beneath was heated and jetted up through the flow, possibly adding to the water content and vesicularity of the flow.

Collapse (or uplift) of the axial zone of a spreading ridge may have occurred after eruption. Such tectonic movements would invalidate the assumption that the depth of collection of submarine lava is the same as the depth of eruption. Collapse of the sea floor from a depth of 2.5 km down to 3.5 km, which is possible on a slow-spreading ridge with a kilometre-deep rift valley, could bring lavas with a vesicularity of 0.9% down to a region where a vesicularity of 0.45% would be reasonably expected.

(2) Since vesicles are ubiquitous in submarine lavas and volatile-rich inclusions are common in phenocrysts, the melts in magma chambers are close to volatile saturation, and some small bubbles are probably present at least in the upper part of shallow magma chambers. If the chamber is non-convecting, such bubbles will tend to rise and collect near the ceiling. If the ceiling is wide and relatively flat, they will be distributed over a wide zone, and most will be incorporated in the solidifying roof rocks marginal to the central region tapped by erupting dykes. If the ceiling is narrow and peaked, then bubbles may accumulate and become enriched in the central apex tapped by dykes.

Other factors might control the distribution of volatiles within the chamber. Processes of liquid diffusion may concentrate volatile components near the cooler ceiling and walls of the chamber or near the ceiling where lower pressure conditions prevail. The content of volatile components in the melt can be enhanced by the digestion and assimilation of hydrothermally altered rocks at the top or upper margins of the chamber and by the crystallisation of early-formed minerals.

The length of feeding dykes, that is, the depth of the magma chamber beneath the ocean floor, will affect the vesicularity of erupted basalt because of the rapid pressure change on eruption. If the volatile content is the same in the upper part of different magma chambers, then lava erupted from a deeper chamber through longer dykes would be more vesicular than that erupted from a shallower chamber, because the greater pressure drop would enhance the growth of existing bubbles and cause exsolution of new bubbles.

(3) The vesicularity of the melt erupted from a magma chamber may be established by differences in the volatile content of magma fed into the bottom of the chamber from the mantle possibly induced by heterogeneities within the mantle, by the mixing of melts from different sources, or by the style of melting.

Discussion

The possibility of lava flows interacting with seawater, thereby increasing vesicularity during eruption and flow on the ocean floor, is believed to be minor because of the general low water content of submarine basalt, which when compared with experimental studies suggests that the melts are undersaturated with water³. Moreover, analyses of ridge basalt from four of the regions included in this study (A2, A5, A9, P2) indicate that the predominant vesicle gas is CO₂, not H₂O. Locally, however, water may be added to basalt melt by the chimney activity described by Ballard¹⁸.

The mechanism of collapse of the ocean floor after eruption of the analysed lava cannot be eliminated and indeed tends to increase the vesicularity in the rifted valleys of slow-spreading ridges. However, the anomalously high vesicularity of some of the sample suites cannot be explained by this process unless collapse amounted to 1,400 m (for suites A8 and A4). Furthermore, there is no evidence in the lavas from suite A5 that lavas of different age show a significant difference in vesicularity.

The higher vesicularity of Atlantic spreading ridge basalt suites compared with those from the Pacific (Fig. 3) suggests that some feature of the shallow storage chambers feeding eruptions may control the vesicularity of erupted lava. Pacific spreading ridges generally spread faster (Fig. 3) and consequently the magma chambers are presumably larger and shallower^{19,20}. The larger chambers are more likely to contain convecting magma which will inhibit the rise of vesicles and collection near the top. Furthermore, the shallower character of fast-spreading chambers would cause them to be tapped by shorter dykes²⁰ subjecting the melt to a smaller pressure drop (as compared with the deeper slow-spreading chambers) thus producing less vesicular erupted lava assuming that the tapped melt had the same volatile content. Finally, the narrow and more peaked aspect of slow-spreading chambers may tend to concentrate rising volatiles towards the top centre of the chamber where they will be tapped by dykes and erupted as more vesicular lava on the ocean floor. In the broader flat-topped chambers of fast-spreading ridges, volatiles will be concentrated over a broader zone of the ceiling, where a higher proportion will be incorporated in the solidifying roof rocks marginal to the fluid central zone tapped by erupting dykes.

Such an explanation cannot account for differences in the vesicularity of Atlantic suites, all of which occur where spreading rates are low and close to a half rate of 1 cm yr⁻¹. However, Cann²⁰ has suggested that because spreading rates of much less than 1 cm yr⁻¹ have not been found, this may mark a lower limit of spreading, where the magma chamber is very narrow¹⁹ and perhaps discontinuous along strike and through time. In such chambers, volatile concentrations of erupted lava may be especially enriched in favourable places by the processes discussed.

The vesicularity contrasts may also be caused by differences in the composition of magma fed into magma chambers from the mantle. Schilling and coworkers^{7,8} have amassed data showing that significant concentrations of trace elements occur in ocean ridge basalts from certain areas where magmas are apparently derived from deep mantle plumes. Such plume-derived lavas are enriched in the halogens, LREE, ⁸⁷Sr/⁸⁶Sr, and other incompatible elements of varying volatility, including K, Ba, Rb and Cs. In the Atlantic, such plume areas include Iceland (A1) and the Azores region (A4, A5, A6, A7), which this study demonstrates are also enriched in vesicles and CO₂. Hence the plume areas may represent sites of upwelling of partial melt from a deep mantle source relatively undepleted in volatiles, and other incompatible, large ion elements. In contrast, the basalts of

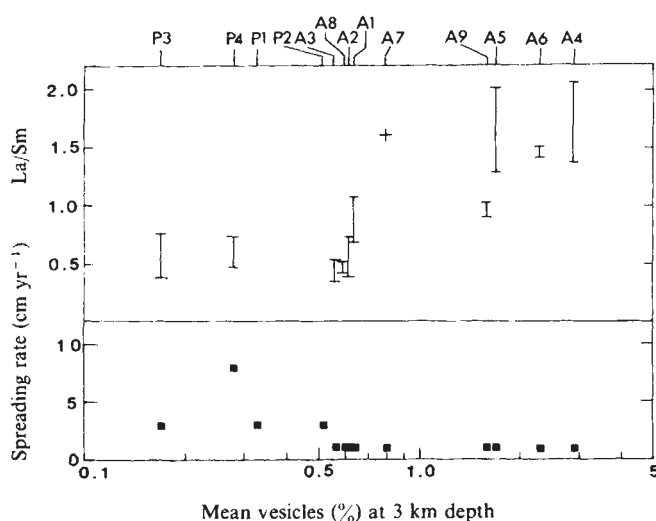


Fig. 3 Logarithmic mean vesicularity adjusted to 3 km depth relative to spreading rate and chondrite normalised La/Sm ratio for 13 suites of mid-ocean ridge basalt.

so-called normal ridge segments such as A8 and the Pacific suites that were sampled are derived from a shallower mantle zone previously depleted in these constituents.

CO₂ is the primary discrete volatile phase in melts erupted on the ocean floor and no doubt exists also as a discrete phase as bubbles in subjacent magma chambers, where it is commonly entrapped in phenocryst minerals. Hence spreading ridge magma chambers which may exist for millions of years will be subjected to a constant flux of upward-streaming CO₂. The extent to which this CO₂ is concentrated in favourable places and is able to concentrate other trace elements and affect isotopic ratios is poorly known. Much of the evidence suggests that the unique chemistry of plume-type basalt is derived from a different, deeper mantle source. But the high CO₂ content of these basalts indicates that their composition may have been substantially altered by the CO₂ flux.

Received 22 May; accepted 5 September 1979.

1. Moore, J. G. *Am. J. Sci.* **263**, 40–52, (1965).
2. Moore, J. G. & Schilling, J. G. *Contr. Miner. Petrol.* **41**, 105–118 (1973).
3. Moore, J. G. *Contr. Miner. Petrol.* **28**, 272–279 (1970).
4. Bryan, W. B. & Moore, J. G. *Bull. geol. Soc. Am.* **88**, 556–570 (1977).
5. Moore, J. G., Normark, W. R., Hess, G. R. & Meyer, C. E., *J. Res. U.S. Geol. Surv.* **5**, 753–759 (1977).
6. Moore, J. G., Batchelder, J. N. & Cunningham, C. G. *J. Volcan. Geotherm. Res.* **2**, 309–327 (1977).
7. Schilling, J. G. *Phil. Trans. R. Soc.* (in the press).
8. Schilling, J. G. *Nature* **242**, 565–571 (1973); *J. geophys. Res.* **80**, 1459–1473 (1975).

It is clear from Fig. 2 that high-vesicle lavas have a much better potential for building islands than do low-vesicle lavas. If we contrast extreme areas with an adjusted average vesicularity at 3 km of 2% with one of 0.30%, then projecting parallel to the Kilauea curve, these values increase at 2 km depth to 4.4 and 0.65%, and at 1 km depth to 17 and 2.5%. If the same amount of melt was erupted from each centre, the high-vesicle lava would build up more rapidly and its rate of buildup would accelerate more rapidly. Hence the presence of islands such as the Azores and Iceland near regions of high-volatile lavas may partly result from this process.

I thank W. B. Bryan (A4, A8, A9), J. B. Corliss (P3), J. Honnorez and E. Bonatti (P4), C. A. Hopson (A6, A7), W. R. Normark (P2) and J. G. Schilling (A1, A2, A3) for samples from the indicated ridge segments; and C. R. Bacon and R. G. Coleman for helpful suggestions.

9. Frey, F. A., Bryan, W. B. & Thompson, G. *J. geophys. Res.* **79**, 5507–5527 (1974).
10. Schilling, J. G. *Earth planet. Sci. Lett.* **25**, 103–115 (1975).
11. White, W. M. & Bryan, W. B. *Bull. geol. Soc. Am.* **88**, 571–576 (1977).
12. Bryan, W. B. & Sargent, D. *Init. Rep. DSDP* **45**, 653–655 (1978).
13. Standigel, H. *Init. Rep. DSDP* (in the press).
14. Thompson, G., Bryan, W. B., Melson, W. G. *EOS* **59**, 405 (1978).
15. Moore, J. G. *Contr. Miner. Petrol.* **28**, 272–279 (1970).
16. Schilling, J. G., Anderson, R. N. & Vogt, P. *Nature* **261**, 108–113 (1976).
17. Schilling, J. G. & Bonatti, E. *Earth planet. Sci. Lett.* **25**, 93–102 (1975).
18. Ballard, R. D. *Prog. Hawaii Symp. Intracate Volcanism and Submarine Volcanism*, 54 (1979).
19. Sleep, N. H. *J. geophys. Res.* **80**, 4037–4042 (1975).
20. Cann, J. R. *R. Astr. Soc. geophys. J.* **39**, 169–187 (1974).

Biomass burning as a source of atmospheric gases CO, H₂, N₂O, NO, CH₃Cl and COS

Paul J. Crutzen, Leroy E. Heidt, Joseph P. Krasnec, Walter H. Pollock & Wolfgang Seiler*

National Center for Atmospheric Research, PO Box 3000, Boulder, Colorado 80307

Biomass burning can contribute extensively to the budgets of several gases which are important in atmospheric chemistry. In several cases the emission is comparable to the technological source. Most burning takes place in the tropics in the dry season and is caused by man's activities.

THE potential importance of deforestation and biomass burning for the atmospheric CO₂ cycle has received much attention and caused some controversy^{1–3}. In this article we will show the probable importance of biomass burning as a trace gas source, which is caused by man's activities in the tropics. We used the results of our global biomass burning analysis⁴ to derive some rough estimates of the sources of the important atmospheric trace gases CO, H₂, CH₄, N₂O, NO_x (NO and NO₂), COS and CH₃Cl from the worldwide burning of biomass. Table 1 shows the results of our study for different activities and ecosystems.

Our approach has been to relate the emission quantities of these gases to those of CO₂ in fire plumes. We have determined such emission ratios during two major forest fires. The first was from a forest fire ~12.5 km south-west of Meeker, Colorado. The crown fire was fuelled by pinon and juniper timber, but most of the material burned consisted of annual and perennial grasses, shrub juniper, and sagebrush in the undergrowth. The dense plume rose to a height of ~4 km before flattening and moving horizontally. Samples were collected in stainless steel containers during flights through the smoke plume at different

altitudes. Background samples were also collected, at plume altitude, 6 km before reaching the sampling area and again when leaving the fire area.

The second fire occurred in the Wild Basin area of the Rocky Mountain National Forest, not far from the Meeker site, in a mature spruce and fir forest in which there was much dead woody material on the ground and much less green vegetation than in the Meeker fire. In this case samples were taken on ground level. CO₂, CO, CH₄, H₂, COS and CH₃Cl were analysed by gas chromatography; N₂O was determined by mass spectrometry. To check for nonlinear gas-chromatographic responses at high concentrations in the samples, a volumetric measurement of CO₂ was also performed.

Some information on emissions from wood burning can also be derived from earlier field and laboratory experiments^{5–9}. The emission ratios are shown in Table 2 which includes only collections with CO₂ concentrations at least 10% above background. At this stage we have not been able to measure gaseous emissions in tropical ecosystems. This is clearly a disadvantage, but it should not invalidate our main conclusions. Biomass burning has previously been considered unimportant as a global source of atmospheric trace gases¹⁰—our analysis shows that this is not the case.

Importance of trace gases in the atmosphere

Some of the trace gases such as CO₂, N₂O and CH₄ considered in this study contribute to the atmospheric greenhouse effect by their absorption of terrestrial thermal radiation¹¹.

Other trace gases such as carbon monoxide (CO) strongly affect the tropospheric concentrations of the highly reactive

* Present address: Max-Planck Institute for Chemistry, Mainz, FRG.

hydroxyl (OH) radical through the reaction



It has, therefore, been proposed that a growth in tropospheric CO from industrial activities would lead to a decrease in tropospheric OH concentrations¹². As the only significant tropospheric sink for many gases (especially hydrocarbons and chlorinated hydrocarbons) is reaction with OH, a rise in the tropospheric CO content would cause enhanced concentrations of these gases in the troposphere and a greater transfer to the stratosphere, with possible effects on stratospheric ozone. Other gases which affect the concentration of OH in the stratosphere include methane (CH₄) and molecular hydrogen (H₂).

Although the oxidation of methane (CH₄) is important, molecular hydrogen (H₂) can also add to the reservoir of stratospheric water vapour. This will affect the formation of stratospheric OH, with further consequences on the chemical and thermal balance of the stratosphere¹³.

Little of the nitric oxide (NO) emitted at ground level can reach the stratosphere because of the efficient precipitative scavenging of its oxidation products NO₂ and HNO₃. The presence of NO in the troposphere affects the formation of ozone, through the oxidation of carbon monoxide, methane and other hydrocarbons¹⁴. It is therefore conceivable that increasing industrial inputs of CO and NO in the atmosphere will lead to increasing levels of tropospheric ozone¹⁴.

As nitrous oxide (N₂O) does not react in the troposphere, it will have a long tropospheric residence time and reach the stratosphere where its oxidation leads to the formation of NO and NO₂. These gases appear in catalytic cycles of reactions leading to less ozone above 25 km and more ozone below this altitude¹⁵.

Carbonyl sulphide (COS) and methyl chloride (CH₃Cl) have similar importance in stratospheric chemistry. During periods of little volcanic activity it is possible that the photodissociation of COS is the main source of sulphur in the stratospheric sulphate layer. Although little is known about the sources of COS there may be a growing anthropogenic input of it¹⁶. The decay of CH₃Cl in the stratosphere releases Cl and ClO, which are extremely efficient in limiting stratospheric ozone by catalytic reactions. An important source of methyl chloride is its emanation from the oceans, but its production by biomass burning has also been proposed¹⁷.

Global source estimates

The data collected in Table 2 show an encouraging uniformity. Individual average emission ratios for several gases do not

deviate more than a factor of 6 from each other. The largest variations in emission ratios are found for N₂O, COS and CH₃Cl. This is not surprising as one may expect these emissions to be particularly dependent on the type of material being burned. For example, the relative nutrient content in the green parts of the biomass is much larger than in stemwood¹⁸. We are not aware of any published emission data for the gases N₂O, H₂, CH₃Cl and COS. With the exception of the lower values obtained by Darley *et al.*⁵ and Boubel *et al.*⁷, the CO/CO₂ emission ratios have all been determined to be in the range of 12–20%.

Because of the uniform CO/CO₂ ratios reported, we can give a reasonable indication of the magnitude of the CO emissions. The same may apply to CH₄ and H₂. It is clear, however, that any estimations are much less reliable for NO_x, N₂O, COS and CH₃Cl. However, we can still indicate the potential importance of biomass burning as a source of these gases to the atmosphere.

If the data on total worldwide CO₂ emissions from wood burning of $2-4 \times 10^{15}$ g C yr⁻¹ from Table 1 are accepted, we can discuss its possible implications for the budgets of CO, H₂, CH₄, NO_x, N₂O, CH₃Cl and COS. The wood burning contribution can then be compared with previously derived estimates of the sources of these gases from other anthropogenic or natural processes. Note that our estimates on the global extent of biomass burning are substantially smaller than those of other workers such as Wong² and Woodwell *et al.*³. We cannot defend the data compiled in Table 1 here due to lack of space, however, acceptance of higher biomass burning rates will clearly lead to larger estimates of trace gas emission rates.

Adopting an average relative CO/CO₂ volume emission factor of 14% from the data collected in Table 2, the average estimated input of CO would be 8.4×10^{14} g CO yr⁻¹, with a range of about $2.4-16.6 \times 10^{14}$ g CO yr⁻¹. Seiler¹⁹ has estimated that the technological input of 6.4×10^{14} g CO yr⁻¹ is the dominant source of atmospheric CO, and a global CO emission of 2.9×10^{14} g yr⁻¹ from burning has been estimated by Bach²⁰. Clearly, the CO input from fires is probably also important for the global CO budget and for the interpretation of the global CO distribution.

With a relative H₂/CO₂ emission ratio of 3.3%, the production of H₂ would amount to 15×10^{12} g H₂ yr⁻¹, with an estimated range of $9-21 \times 10^{12}$ g H₂ yr⁻¹. Schmidt²¹ estimated the total worldwide source of H₂ to be 36.5×10^{12} g yr⁻¹, including an anthropogenic contribution of 25.5×10^{12} g yr⁻¹. Although our analysis shows that fires are probably important no such contribution was considered in his budget.

Assuming an average CH₄/CO₂ emission ratio factor of 1.6%, the global source of CH₄ can be calculated as

Table 1 Summary of data for the annually burned area and biomass

Activity	Burned and/or cleared area	Total biomass cleared	Biomass exposed to fire	Annually burned biomass	Dead below-ground biomass	Dead unburned above-ground biomass
Burning due to shifting agriculture	21–62 (41)	31–92 (62)	24–72 (48)	9–25 (17)	7–20 (14)	16–72 (44)
Deforestation due to population increase and colonisation	8.8–15.1 (12.0)	20–33 (26.5)	16–25 (20.5)	5.5–8.8 (7.2)	4.0–8.0 (6.0)	10.5–16.0 (13.3)
Burning of savanna and bushland	(600)		12.2–23.8 (18)	4.8–19 (11.9)	8–16 (12)	2.4–4.8 (3.6)
Wildfires in temperate forests	3.0–5.0 (4.1)	10.5–17.5 (14.0)	7.7–12.8 (10.3)	1.5–2.6 (2.1)	2.8–4.7 (3.8)	6.2–10.2 (8.2)
Prescribed fires in temperate forests	2.0–3.0 (2.5)	1.2–1.8 (1.5)	0.3–0.5 (0.4)	0.1–0.2 (0.2)	0.6–0.9 (0.8)	0.2–0.3 (0.3)
Wild fires in boreal forests	1.0–1.5 (1.3)	2.5–3.8 (3.2)	1.8–2.7 (2.3)	0.4–0.6 (0.5)	0.7–1.1 (0.9)	1.4–2.1 (1.8)
Burning of industrial wood and fuel wood		31–32 (31.5)	11–12 (11.5)	10–11 (10.5)	5.5	1*
Burning of agricultural wastes			19–23 (21)	17–21 (19)	27–31 (29)	1.9–2.3 (2.1)
Total	630–690 (660)	130–250 (180)	92–172 (132)	48–88 (68)	56–87 (72)	40–109 (74)

Units 100 Tg dry matter and 10⁶ hectares; to convert dry matter to carbon multiply by 0.45. Data in parentheses represent average values.

* Excluding wood used in long lasting structures.

Table 2 Compilation of product yields by volume relative to that of CO₂ from different studies

Excess of trace gas concentrations compared to excess CO ₂ over background	H ₂ (%)	CH ₄ (%)	CO (%)	N ₂ O (%)	NO _x (%)	COS (×10 ⁻⁶)	CH ₃ Cl (×10 ⁻⁶)
Meeker forest fire (12 July 1977)							
Average (four observations)	3.3	2.1	12.4	0.38			
Range	2.9–3.5	1.6–2.5	11.2–13.5	0.23–0.50			
Wild Basin fire (20 September 1978)							
Average (seven observations)		2.2	19.9	0.06		15.8	23.4
Range		1.0–3.4	15.8–25.1	0.02–0.08		5.4–28.6	4.4–57.2
Agricultural wastes ⁵		1.2	6.2				
		0.15–4.6	3.3–15.8				
Landscape refuse ⁶			14.6		0.28		
Grass, stubble and straw ⁷							
Average			6.3				
Range			2.9–14.9				
Eight pine slash fires ⁸ (average)			19.7		0.65(NO + NO ₂)		
Wood burning in fire places ⁹ (average)			18.0				
Average ratios used for source estimates	3.3	1.6	14	0.22	0.47	15.8	23.4
Range	2.9–3.5	1.0–2.2	5.5–21.6	0.02–0.50	0.28–0.65	5.4–28.6	4.4–57.2

The forest fire plume data were obtained from wild fire observations. The remaining data were obtained from controlled fires or laboratory measurements.

6×10^{13} g yr⁻¹ with a range of $2.5\text{--}11 \times 10^{13}$ g CH₄ yr⁻¹. The combustion vegetation could contribute to the CH₄ budget of the atmosphere, which has an estimated input of $40\text{--}83 \times 10^{13}$ g CH₄ yr⁻¹ (ref. 22). However, methane is not the only hydrocarbon gas emitted from the burning of vegetation: emissions of many reactive hydrocarbons, including acetylene (C₂H₂) have also been detected⁵ and for some of these gases wood burning may be an important source.

As explained above, the derivation of global source estimates for N₂O, NO_x, COS and CH₃Cl is not trivial. The resulting fluxes which we will derive for these gases are, therefore, less reliable than those derived for CO, H₂ and CH₄. The simplest way of estimating the flux would be to apply the same procedure as for CO, H₂ and CH₄. With an approximate N₂O/CO₂ emission factor of 0.22%, the estimated nitrous oxide source would be 20×10^{12} g N₂O yr⁻¹. Similar calculations would yield a source of 14×10^{12} g N yr⁻¹ for NO_x, 1.1×10^{11} g S yr⁻¹ for COS, and 1.9×10^{11} g Cl yr⁻¹ for CH₃Cl.

Although this approach indicates the potential importance of emission strengths, it is a dubious estimation. An upper limit to the NO_x emissions can be obtained by estimating the mass of bound nitrogen in the burned plant material and by assuming that almost all bound nitrogen is emitted as NO_x—an assumption consistent with other experimental studies^{8,23,24}. The average of eight experimental slash fires gave relative volume emission ratios NO_x/CO₂ of 4% for duff and 2.1% for needles. These ratios are close to the values that can be expected if essentially all bound nitrogen in the plant material were volatilised as NO_x. No NO_x emissions were detected during the combustion of cellulose materials, presumably because burning temperatures were too low to allow appreciable NO_x production from atmospheric N₂ and O₂. The relative volume yield NO_x/CO₂ from an entire fire (in which about 55% of all available fuel was consumed) was estimated to be 0.65%⁸. In living plants the nutrients N and S are mainly concentrated in those parts which are most easily burned, such as leaves, small twigs and bark. Before burning, however, much plant tissue is dry and dead, and has actually lost nutrients. Using the information in our Table 1 and Tables 53 and 54 in the compilation by Rodin and Bazilevich¹⁸, the average maximum global N/C ratio in the burned biomass can be roughly estimated to be between 1.5 and 2.5%. Given this information we derive a maximum global average NO_x emission of 50×10^{12} g N yr⁻¹ from biomass burning, with a range of $20\text{--}100 \times 10^{12}$ g N yr⁻¹ if most bound nitrogen is indeed emitted as NO_x. The mean estimate is about equal to that mentioned previously by Delwiche and Likens²⁵.

The assumption that all fixed nitrogen is released as NO_x is far from established. A study by Clements and McMahon²⁶ confirms that most of the NO_x released during burning arises

from fuel nitrogen. They conclude that environmentally significant amounts of NO_x are formed by the burning of forest fuels. However, this study also indicated that the NO_x yield is not 100% and that it actually increases with the nitrogen content of the fuel: a rough average of the NO_x yield observed in their studies is about 30%. From a mass-balance perspective it is therefore conceivable that other trace gases besides NO_x are released by biomass burning. These could include N₂O, NH₃ and HCN. NH₃ is an important gas involved in the atmospheric nitrogen cycle. HCN has not so far been observed in the atmosphere, but it could be rather stable as it is not removed photochemically in the troposphere.

If we assume that our maximum observed N₂O/CO₂ emission ratio of ~0.4% is produced from the burning of the nutrient-rich portions of the vegetation (such as the needles and leaves, which have an average N/C composition ratio of 4–6%), we may guess that the N₂O volume emissions could be ~8% of those of NO_x. Consequently we may estimate an average global N₂O source of $\sim 13 \times 10^{12}$ g N₂O yr⁻¹. Finally, comparing the relative emission ratios of COS and CH₃Cl to those of N₂O obtained from the Wild Basin fire in Colorado, the global emission rate of COS can be roughly extrapolated to be 2.4×10^{11} g S yr⁻¹ (3×10^7 molecules cm⁻² s⁻¹ at the Earth's surface) and that of CH₃Cl to be 4.2×10^{11} g Cl yr⁻¹ ($\approx 4 \times 10^7$ molecules cm⁻² s⁻¹). Judging from these estimates, biomass burning could produce much more COS than is destroyed in the stratosphere¹⁶. This may indicate the presence of another sink for COS in the troposphere or at the Earth's surface. On the other hand, the production of CH₃Cl from biomass burning seems to be an order of magnitude lower than its destruction in the troposphere by reaction with OH²⁷. Therefore, from this limited analysis it seems that the oceanic source of CH₃Cl should be more important¹⁷.

The indicated emissions of NO_x and N₂O both seem very important from a global perspective. A NO_x source strength of several times 10^{13} g N yr⁻¹ is comparable to or larger than the other two major sources of NO_x (industrial processes²⁸ and lightning²⁹). The production of NO_x by the burning of vegetation during the dry season has been clearly demonstrated by Lewis and Weibezahn³⁰, who measured an increase in rainfall acidity by 10⁴ from the beginning to the end of the dry season in the Aragua Valley in Venezuela. According to these authors the release of NO_x by seasonal vegetation burning and its conversion to HNO₃ were responsible for this phenomenon. The S/N ratios in plant material are too low for H₂SO₄ to be nearly as important as HNO₃ in the acidification of the precipitation. The observations by Lewis and Weibezahn³⁰ supported our method of extrapolation from mid-latitude to tropical conditions.

The indicated production rates of N₂O by burning are comparable to the removal rate in the stratosphere, which is still

the only well-documented sink for nitrous oxide. The large oceanic source of N_2O once derived by Hahn³¹ is now strongly disputed and more recent observations point to a much smaller source^{32,33}. It seems, therefore, that vegetation burning as a source of N_2O could be of a similar substantial importance.

Note that in our source calculations for the atmospheric trace gases, we did not consider the potential release from the heating of the topsoil organic matter or from the $40\text{--}80 \times 10^{14}$ g C of matter which is exposed to fire but left behind as dead, unburned above-ground biomass (see Table 1). The topsoil organic matter is especially rich in nutrients and may make important contributions to the cycling of atmospheric trace gases and nutrient elements. The latter has already been pointed out by Lewis³⁴.

The analysis of emissions from the burning of vegetation has also revealed substantial yields of aldehydes, hydrogen peroxide (G. Kok, personal communication), and reactive and oxygenated hydrocarbons^{7,35,36}. Because of the simultaneous production of NO, there may be a significant production of

ozone in the fire-affected air. Such production of ozone has been observed³⁷.

Despite the limited amount of observations, we must conclude that biomass burning, especially in the tropics where the rates of biomass production and biomass burning are unparalleled, has the potential to contribute in an important way to the global budgets of several major atmospheric trace gases. We also need to consider that tropical emissions occur in a photochemically very active and dynamically important region, in which substantial transfer of tropospheric air to the stratosphere takes place.

Observations of biomass burning in the tropics are needed which will enable us to assess far more reliably the role of biomass burning in biogeochemical cycles and in atmospheric chemistry. This research is especially important in view of the increasing use of forest resources in the developing countries.

We thank Drs W. Lewis and C. McMahon for valuable comments. The National Center for Atmospheric Research is sponsored by the NSF.

Received 1 June; accepted 20 September 1979.

1. Wong, C. S. *Mar. Pollut. Bull.* **9**, 257–264 (1978).
2. Woodwell, G. M. *et al. Science* **199**, 141–146 (1978).
3. Bolin, B. *Science* **196**, 613–615 (1977).
4. Seiler, W. & Crutzen, P. J. *Climatic Change* (in the press).
5. Darley, E. F., Burleson, F. R., Mateer, E. H. & Middleton, J. T. *Air Pollut. Control Ass. J.* **11**, 685–690 (1966).
6. Gerstle, R. W. & Kemnitz, D. A. *Air Pollut. Control Ass. J.* **17**, 324–327 (1967).
7. Boubel, R. W., Darley, E. F. & Schuck, E. A. *Air Pollut. Control Ass. J.* **19**, 497–500 (1961).
8. Malte, P. C. *Washington State Univ. Bull.* 339 (Pullman, Washington, 1975).
9. Short, J. E. *Air Quality Source Testing Rep.* No. 74–1 (Department of Environmental Health, Albuquerque, 1974).
10. Robinson, E. & Robbins, R. C. in *Global Effects of Environmental Pollution* (ed. Singer, W. F.) (Reidel, Dordrecht, 1970).
11. Wang, W. C., Yung, Y. L., Laci, A. A., Mo, T. & Hansen, J. E. *Science* **194**, 685–690 (1976).
12. Wofsy, S. C. *A. Rev. Earth planet. Sci.* **4**, 441–469 (1976).
13. Nicolet, M. *Rev. geophys. Space Phys.* **13**, 593 (1975).
14. Fishman, J. & Crutzen, P. J. *Nature* **274**, 855–858 (1978).
15. Duewer, W. H., Wuebbles, D. J., Elissaesser, H. W. & Chang, J. S. *J. geophys. Res.* **82**, 935–942 (1977).
16. Crutzen, P. J. *Geophys. Res. Lett.* **3**, 73–76 (1976).
17. Lovelock, J. E. *Nature* **256**, 193–197 (1975).
18. Rodin, L. Ye & Bazilevich, N. I. *Production and Mineral Cycling in Terrestrial Vegetation* (Oliver and Boyd, Edinburgh, 1966).
19. Seiler, W. *Tellus* **26**, 116 (1974).
20. Bach, W. *Bonner met. Abh.* **24**, 1–51 (1976).
21. Schmidt, H. U. *Tellus* **26**, 78–90 (1974).
22. Ehhalt, D. H. *Tellus* **26**, 58 (1974).
23. Isichei, A. O. & Sanford, W. W. *SCOPE/UNEP Workshop on Nitrogen Cycling in West African Ecosystem* (IITA, Ibadan, 1978).
24. Nye, P. H., & Greenland, D. J. *The Soil Under Shifting Cultivation* (Tech. Commun. No. 51, Commonwealth Bureau of Soils, Harpenden, 1960).
25. Delwiche, C. C. & Likens, G. E. in *Global Chemical Cycles and Their Alterations by Man* (ed. Stumm, W.) 73–88 (Dahle Konferenzen, Berlin, 1977).
26. Clements, H. B. & McMahon, C. K. *Thermochim. Acta* (submitted).
27. Chang, J. S. & Penner, J. E. *Atmos. Envir.* **12**, 1867–1873 (1978).
28. Söderlund, R. & Svensson, B. H. *Ecol. Bull., Stockholm* **22**, 23 (1976).
29. Chameides, W. L., Stedman, D. H., Dickerson, R., Rusch, D. W. & Ciccone, R. J. *J. atmos. Sci.* **34**, 143 (1977).
30. Lewis, W. M., Jr & Weibezahn, F. H. *Science* (submitted).
31. Hahn, J. *Tellus* **26**, 160 (1974).
32. Cohen, Y. & Gordon, L. I. *J. geophys. Res.* **84**, 347–354 (1978).
33. Weiss, R. F. *EOS* **59**, 1101 (1978).
34. Lewis, W. M., Jr in *Mineral Cycling in Southeastern Ecosystems* (eds. Howell, F. G., Gentry, J. B. & Smith, M. H.) 833–846 (US ERDA, 1975).
35. Feldstein, N., Duckworth, S., Wohlers, H. C. & Linsky, B. *Air Pollut. Control Ass. J.* **13**, 542–545 (1963).
36. Fritschen, L. *et al.* USDA Forest Service Res. Pap. PNW-97 (1970).
37. Evans, L. F., Weeks, I. A., Eccleston, A. J. & Packham, D. R. *Envir. Sci. Technol.* **11**, 896–900 (1977).

A modified RNA polymerase transcribes a cloned gene under sporulation control in *Bacillus subtilis*

William G. Haldenwang & Richard Losick

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

A modified form of RNA polymerase from Bacillus subtilis selectively transcribes a cloned gene under early sporulation control. This RNA polymerase lacks σ factor but contains a newly identified subunit of molecular weight 37,000, termed P³⁷. P³⁷ could be a regulatory protein that controls, at least in part, an early stage of spore development.

THE formation of dormant endospores in *Bacillus subtilis* is a simple developmental process that proceeds through a series of well defined morphological stages (for reviews see refs 1, 2). During the earliest developmental stages, the sporulating cell partitions itself by invagination of the plasma membrane into a forespore compartment and a mother cell or sporangium. Then, after engulfment of the forespore by the mother cell membrane, the developing spore acquires a cortex and a tough protein coat. Finally, a mature, dormant spore is liberated by lysis of the

mother cell. Mutations in a variety of sporulation or *spo* genes arrest this process at different stages of spore development¹.

What mechanisms govern the expression of sporulation genes during this developmental sequence? To help answer this question, this laboratory previously cloned a *B. subtilis* gene whose transcription is activated at an early stage of spore development³. This gene, which maps near the origin of replication of the *B. subtilis* chromosome (between *spo0J* and *spoIIE20*, ref. 4, and W.G.H., C. Banner, J. F. Ollington, R.L., and A. L. Sonenshein, unpublished results) encodes an RNA of about 400 bases and referred to as the '0.4-kilobase gene'. This gene is known to be under sporulation control because its transcription is severely restricted in mutants blocked at the earliest stage of development (*spo0A*, *spo0B*, *spo0E*, *spo0F* and *spo0H* mutants; ref. 3 and unpublished results). Here we report the isolation from early sporulating cells of a form of *B. subtilis* RNA polymerase that transcribes this developmentally regulated gene *in vitro*.

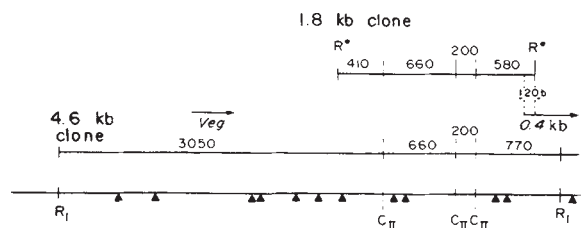


Fig. 1 Endonuclease restriction map of cloned *B. subtilis* DNA in the vicinity of the 0.4-kilobase gene. The lower line is a map of the cleavage sites for the restriction enzymes *HpaII* (▲), *EcoRI* (as indicated) and *HincII* (as indicated) and is a composite of the maps of several overlapping cloned DNAs. The middle line is a map of one cloned DNA of 4.6 kilobases that terminates at an *EcoRI* site within the 0.4-kilobase gene, and the top line is a map of a cloned 1.8-kilobase DNA that terminates at an *EcoRI** cleavage site within the 0.4-kilobase gene. The two heavy arrows show the location and direction of transcription of the 0.4-kilobase gene and the vegetative (*veg*) gene. The numbers on the middle and top lines show the size in base pairs of fragments generated by *HincII* cleavage of the 4.6- and 1.8-kilobase DNAs (see Figs 2, 4). The dashed lines show the position of the 120 base run-off RNA (see Fig. 4). Documentation for the information in this figure will be presented elsewhere (J. F. Ollington, W.G.H. and R.L., manuscript in preparation).

Detection of a specific transcribing activity

Figure 1 presents an endonuclease restriction map of cloned *B. subtilis* DNA in the vicinity of the 0.4-kilobase gene. The 0.4-kilobase gene, as depicted, is transcribed from left to right with its startpoint for transcription located about 300 base pairs from an internal *EcoRI* cleavage site (J. F. Ollington, W.G.H. and R.L., in preparation). At 2.7 kilobases to the left of the 0.4-kilobase gene, there is a sequence that is transcribed during growth and sporulation and referred to as the 'vegetative' gene.

We devised a specific hybridisation assay to search for an activity that would transcribe the 0.4-kilobase gene. A hybrid plasmid containing a 4.6-kilobase insert defined by the two *EcoRI* sites in the map was cleaved with the restriction enzymes *HincII* and *EcoRI*. This generated fragments of 3,050 and 2,200 base pairs that contained the vegetative gene and a 770-base pair fragment containing the promoter-proximal portion of the 0.4-kilobase gene as well as several other fragments of *B. subtilis* and vector DNA. (The 2,200-base pair DNA is a secondary cleavage product of the 3,050-base pair fragment that is generated from an *EcoRI** site near the left end of the 4.6-kilobase DNA; see Fig. 2 legend). These fragments were separated electrophoretically and transferred to nitrocellulose by the method of Southern⁵. Radioactive RNA from cells pulse-labelled early in sporulation hybridised to fragments containing the vegetative gene and the 0.4-kilobase gene (ref. 3 and J. F. Ollington, W.G.H. and R.L., in preparation). In contrast, radioactive RNA from several different stage 0-blocked mutants annealed only to 3,050- and 2,200-base pair fragments containing the vegetative gene.

To test for transcription of the 0.4-kilobase gene *in vitro*, we hybridised radioactive RNA copied from hybrid plasmid DNA to the electrophoretically separated fragments. The experiment of Fig. 2 shows that RNA polymerase holoenzyme (lane 1) and core RNA polymerase (containing a trace of σ polypeptide) purified from vegetative cells (lane 2) selectively transcribed the vegetative gene region of the hybrid plasmid although neither form of polymerase copied RNA from the 770-base pair region that contained the 0.4-kilobase gene. Thus, purified vegetative RNA polymerase failed to recognise the sporulation-controlled sequence.

To search for a form of RNA polymerase that would copy the 0.4-kilobase gene, we examined extracts of cells collected early (T_1) in sporulation. We identified such an activity in enzyme partially purified by phase partitioning, gel filtration and phosphocellulose chromatography. Lane 3 of Fig. 2 shows that partially purified sporulation enzyme actively copied RNA from the 770-base pair region as well as from the vegetative gene-containing fragments. Some transcription of two plasmid vector

fragments (2,400 and 2,210 base pairs) could also be detected. Transcription of hybrid plasmid DNA was not random, as we could detect no hybridisation to the 660- and 200-base pair fragments preceding the 0.4-kilobase gene or to a vector fragment of 950 base pairs.

As a further test of recognition of the 0.4-kilobase gene, we examined the strand selectivity of transcription from the 770-base pair region. DNA strands of the 770-base pair fragment were separated by gel electrophoresis of denatured DNA and then transferred by the Southern⁵ procedure to nitrocellulose. Hybridisation of radioactive 0.4-kilobase RNA synthesised *in vivo* identified the slow-migrating DNA as the DNA coding strand (Fig. 3, lane 3). (Other work showing that the 5'-end of the slow-migrating strand is at the *EcoRI* end of the 770-base pair fragment established the direction of transcription as shown in Fig. 1). Figure 3 (lane 2) shows that RNA synthesised *in vitro* from hybrid plasmid DNA annealed selectively to the slow-migrating strand. Combining this result with the result of Fig. 2, we conclude that the RNA polymerase activity from early sporulating cells initiated transcription within the 770-base pair region and copied RNA asymmetrically from the coding strand of the 0.4-kilobase gene.

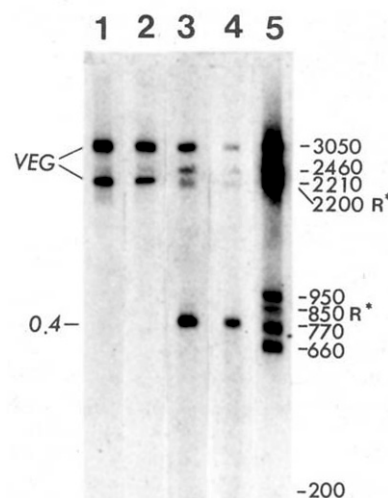


Fig. 2 Southern hybridisations to electrophoretically separated restriction fragments of hybrid plasmid DNA. A pMB9 hybrid plasmid containing the 4.6-kilobase insert of *B. subtilis* DNA³ described in Fig. 1 was cleaved with the restriction enzymes *EcoRI* and *HincII*, generating plasmid vector fragments of 2,460, 2,210 and 950 base pairs and *B. subtilis* DNA fragments of 3,050, 770, 660 and 200 base pairs. (In addition, *EcoRI* cleavage generated small amounts of two fragments of 2,200 and 850 base pairs that arise by secondary cleavage of the *B. subtilis* 3,050-base pair fragment at an *EcoRI** site located 850 base pairs from the left end of the 4.6-kilobase DNA. *EcoRI** is an altered form of *EcoRI* of reduced recognition specificity¹¹.) The fragments were separated electrophoretically and transferred to nitrocellulose by ascending chromatography as described by Southern⁵. The nitrocellulose strips (0.5 μ g DNA per strip) were then incubated in hybridisation conditions (5 $\times$ SSC, 50% formamide; 18 h at 37 $^{\circ}$ C) with radioactive RNA that had been copied *in vitro* from hybrid plasmid DNA. RNA was synthesised *in vitro* by the following protocol: 0.1 units [a unit incorporates 1 nmol of AMP into RNA with poly(dA-T) as template] of RNA polymerase holoenzyme¹² (lane 1), core RNA polymerase¹² (lane 2), phosphocellulose-purified sporulation polymerase (fraction 20 of Fig. 5; lane 3) or DNA-cellulose-purified sporulation polymerase (fraction 36 of Fig. 6, lane 4) were separately allowed to pre-bind for 10 min at 37 $^{\circ}$ C with 2.5 μ g of pMB9-4.6-kilobase hybrid plasmid DNA (representing an enzyme to template ratio of about 0.5–1.0) in 50- μ l reaction mixtures⁶ lacking ribonucleotides but containing 20% glycerol. Next, ribonucleotides (0.15mM CTP, GTP and ATP and 0.4 μ M (5 μ Ci) [α -³²P] UTP) were added. After 4 min incubation, unlabelled UTP (0.15 mM) was added. The reactions were stopped after 2 min and RNA was isolated for hybridisation as described elsewhere⁶. After the hybridisation reactions were completed, the Southern strips were washed, treated with RNase and exposed to X-ray film (Kodak SB-5) to visualise hybrids. Lane 5 shows the position of plasmid vector and *B. subtilis* DNA fragments as visualised by annealing a nitrocellulose strip (that had been treated by the Denhardt¹³ procedure) with hybrid plasmid DNA that had been radioactively labelled *in vitro* by the 'nick-translation' activity¹⁴ of DNA polymerase I.

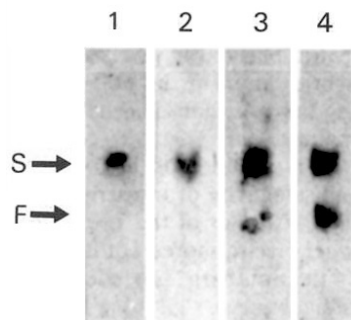


Fig. 3 DNA strand selectivity of transcription. The 770-base pair fragment (containing the 0.4-kilobase gene) was isolated by electrophoresis of pMB9–4.6-kilobase hybrid plasmid DNA that had been cleaved with *HincII* and *EcoRI* (see Fig. 1). The strands of the 770-base pair fragment were then separated by electrophoresis of denatured DNA through a 5% polyacrylamide gel as described by Hayward¹⁵. The separated DNA strands (0.05 µg per strip) were then transferred by the Southern³ procedure to nitrocellulose. Nitrocellulose strips containing separated DNA strands were then incubated in hybridisation conditions (see Fig. 1 legend) with: lane 1, 120-base run-off RNA from the experiment of Fig. 4; lane 2, RNA transcribed *in vitro* from pMB9–4.6-kilobase hybrid plasmid DNA by phosphocellulose-purified sporulation polymerase from the experiment of Fig. 2; lane 3, *in vivo* synthesised 0.4-kilobase RNA isolated from ³²P-labelled sporulating cells as described previously³. After hybridisation, the strips were washed, treated with RNase and exposed to X-ray film to visualise hybrids. Lane 4 shows the position of the slow-migrating (S) and fast-migrating (F) strands as visualised by annealing a nitrocellulose strip with radioactively labelled hybrid plasmid DNA (see Fig. 2 legend).

Initiation of transcription at a unique site

Does sporulation enzyme initiate transcription at a unique site within the 770-base pair region? To answer this question we used a cloned subfragment of 1.8 kilobases that contained the promoter-proximal region of the 0.4-kilobase gene and entirely lacked the vegetative gene. The right-hand end of the 1.8-kilobase fragment is an *EcoRI** cleavage site that falls within the 0.4-kilobase gene at 100–150 base pairs from the startpoint of transcription. We therefore reasoned that, if polymerase was initiating at a unique site near the promoter for the 0.4-kilobase gene, the RNA polymerase from sporulating cells with 1.8-kilobase DNA as template should generate a discrete-sized 'run-off' RNA of 100–150 bases.

This expectation was confirmed by the finding, shown in Fig. 4, that sporulation enzyme generated an RNA of 120 bases from hybrid plasmid DNA that had been cleaved with restriction enzyme to free the 1.8-kilobase insert from the vector. The size of this transcript was apparently dependent on run-off termination at an end of the cut DNA template, as the 120-base RNA was not generated during transcription of uncut plasmid template (Fig. 4). To prove that the 120-base RNA was copied from the 0.4-kilobase region of the hybrid plasmid, *in vitro* synthesised transcript was eluted from a slice of polyacrylamide gel containing the RNA band and from surrounding slices and annealed to electrophoretically separated fragments of the hybrid plasmid. Figure 4b shows that hybridisation to the 580-base pair (Fig. 1) fragment containing the 0.4-kilobase gene promoter region was only observed for the slice that contained the 120-base RNA. Finally, hybridisation to separated DNA strands (Fig. 3, lane 1) showed that the 120-base RNA was complementary to the DNA coding strand for the 0.4-kilobase gene. Thus, we conclude that sporulation enzyme initiated transcription at a unique site close to or identical with the promoter for the 0.4-kilobase gene.

Isolation of a modified RNA polymerase

Using cloned DNAs as probes for specific RNA synthesis, we extensively purified a modified form of RNA polymerase that transcribes the 0.4-kilobase gene. Figure 5a shows the polypeptide profile of proteins eluted from phosphocellulose with a

linear salt gradient. As judged by hybridisation of *in vitro* synthesised RNA to the 770-base pair fragment (Fig. 5b) and by the production of the 120-base RNA (Fig. 5c), transcription of the 0.4-kilobase gene was preferentially associated with RNA polymerase eluting just past the centre of the gradient at 0.43 M KCl.

Although RNA polymerase was impure at this stage, the subunits of core RNA polymerase ($\beta'\beta\alpha_2$) were readily visualised in fractions across the gradient. In addition, those fractions that transcribed the 0.4-kilobase gene seemed to contain preferentially polypeptides of molecular weight (MW) 37,000 (P^{37}) and 36,000 (P^{36}).

Fractions 20–22 (containing RNA polymerase that preferentially transcribed the 0.4-kilobase gene) were pooled and subjected to DNA-cellulose chromatography. Gradient elution resolved two forms of RNA polymerase (Fig. 6a). In addition to the subunits of core RNA polymerase, proteins eluting at lower ionic strength (0.30 M KCl) included the P^{36} polypeptide and a

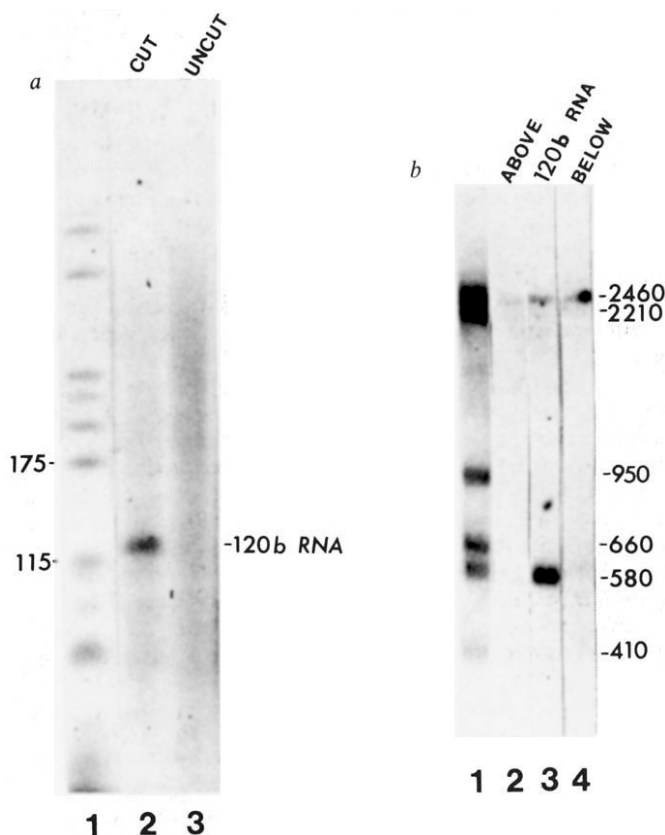


Fig. 4 Synthesis of a 120-base run-off RNA. *a*, RNA was copied *in vitro* using 0.2 units of phosphocellulose-purified sporulation polymerase (fraction 20 of Fig. 5) from a pMB9 hybrid plasmid containing the 1.8-kilobase *EcoRI** fragment described in Fig. 1 legend. The conditions of RNA synthesis were as described in Fig. 2 legend except that heparin (30 µg) was added 30 s after the addition of ribonucleotides to block re-initiation of transcription. The template (2.5 µg) for *in vitro* transcription was either uncut hybrid plasmid DNA (lane 2) or hybrid plasmid DNA that had been cleaved with *EcoRI* to separate the 1.8-kilobase insert from vector DNA (lane 3). (Even though the 1.8-kilobase DNA was defined by two *EcoRI** restriction sites, when cloned into the *EcoRI* site of pMB9, the resulting *EcoRI*–*EcoRI** hybrid site was readily cleaved in *EcoRI* conditions.) *In vitro* synthesised RNA was then subjected to electrophoresis through a 5% polyacrylamide gel containing 7 M urea. As MW standards, lane 1 displays denatured *HaeIII* fragments of ³²P-labelled pMB9 DNA. The numbers on the left of *a* give the size in bases of two fragments. After electrophoresis, the polyacrylamide gel was exposed to X-ray film (Kodak SB-5) to visualise *in vitro* synthesised RNA and the radioactive standards. *b*, After visualisation of the 120-base RNA (*a*, lane 2) 0.5-cm slices were cut from the polyacrylamide gel from positions above (lane 2), coincident with (lane 3) and below (lane 4) the run-off RNA. The slices were crushed and then incubated in hybridisation conditions (see Fig. 2 legend) with Southern strips containing electrophoretically separated fragments of pMB9–1.8-kilobase hybrid plasmid DNA that had been cleaved with *EcoRI* and *HincII* (see Fig. 1). These *EcoRI*–*HincII* fragments were visualised in lane 1 of *b* by reaction of a Southern strip with ³²P-labelled hybrid plasmid DNA (see Fig. 2 legend).

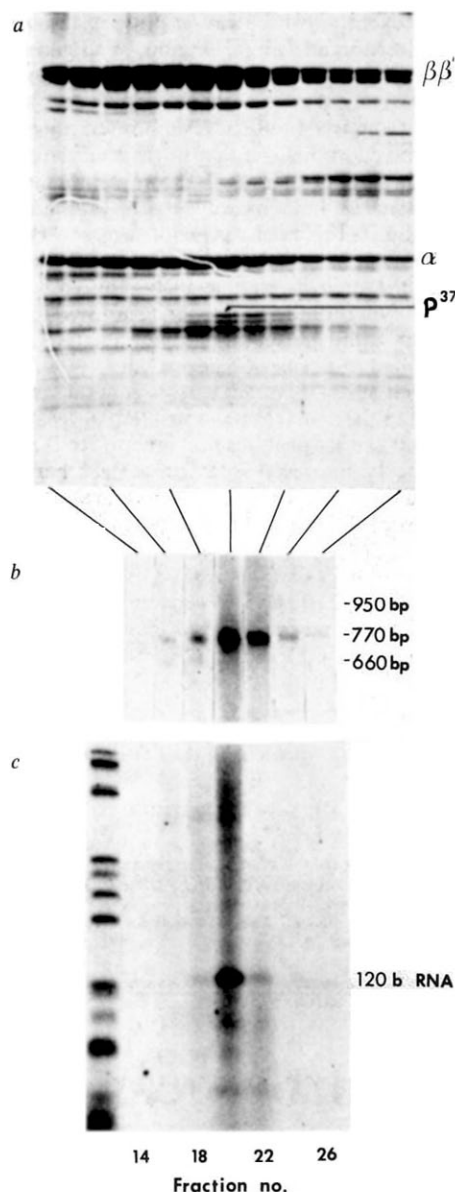


Fig. 5 Gradient elution of RNA polymerase from phosphocellulose. 100 g of *B. subtilis* cells (strain SMY) were collected at the first hour (T_1) of sporulation in Difco sporulation medium¹⁶. The cells were disrupted by two passages through a French pressure cell (8–10,000 p.s.i.) and RNA polymerase was partially purified by phase partitioning and gel filtration as described by Shorenstein and R.L.¹². Next, 10 mg of gel-filtration-purified enzyme was applied to a phosphocellulose column (5 × 1.5 cm) and eluted with a 0.2–0.8 M linear gradient of KCl in 100 ml of buffer C (0.05 M Tris, pH 8.0, 20% glycerol, 0.3 mM dithiothreitol, 1 mM EDTA). Fractions of 2 ml were collected. *a*, Samples of 100 μ l from each fraction were subjected to electrophoresis through a 23-cm-long slab gel containing SDS in a Tris-glycine buffer and a 10–20% gradient of acrylamide¹⁷. The gel was stained with Coomassie brilliant blue. *b*, RNA was generated *in vitro* from pMB9–4.6-kilobase plasmid DNA in reaction mixtures containing 2.5- μ l samples from every other fraction along the gradient and annealed with Southern strips containing *Eco*RI–*Hinc*II fragments of hybrid plasmid DNA. Hybrids were visualised by autoradiography. The panel displays hybridisation in the region of the Southern strips containing *B. subtilis* fragments of 770 and 660 base pairs and a pMB9 fragment of 950 base pairs. Methods for this experiment were described in Fig. 2 legend. The 120-base run-off RNA was synthesised as described in Fig. 4 legend in reaction mixtures containing 2.5- μ l samples from every other fraction from the gradient. The products of *in vitro* RNA synthesis are displayed by polyacrylamide gel electrophoresis and autoradiography. MW standards (see Fig. 4) are displayed on the left.

high molecular weight polypeptide. Proteins eluting at the higher ionic strength (0.41 M KCl) included P^{37} , and this polypeptide was present in a stoichiometry of approximately one per RNA polymerase core molecule. As measured by hybridisation to the 770-base pair fragment (Fig. 6*b*) and by the synthesis of the 120-base RNA (Fig. 6*c*), transcription of the 0.4-kilobase gene was strongly correlated with fractions containing P^{37} . Moreover, RNA polymerase in P^{37} -containing fractions exhibited a marked preference for the 0.4-kilobase gene; as shown in the hybridisation experiment of Fig. 2 (lane 4), enzyme in the peak fraction (fraction 36) for P^{37} content copied RNA with high selectivity from the sporulation-controlled sequence and generated less RNA from the vegetative gene or other regions of the hybrid plasmid.

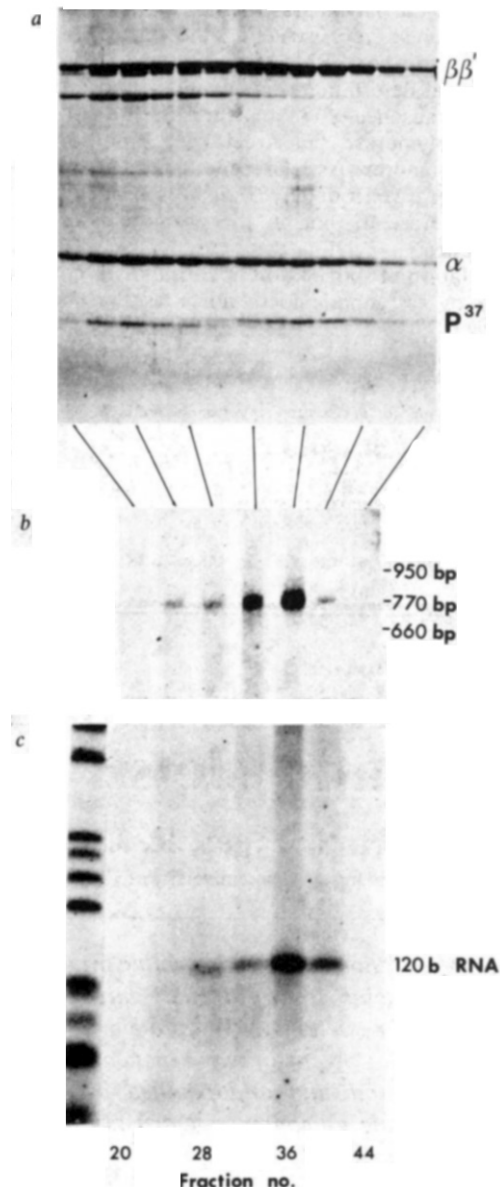


Fig. 6 Gradient elution of RNA polymerase from DNA-cellulose. Phosphocellulose-purified RNA polymerase in fractions 20–22 from the experiment of Fig. 5 were pooled and applied to a calf thymus DNA cellulose column (2 × 1 cm). Enzyme was eluted with a 0.2–0.7 M linear gradient of KCl in 50 ml of buffer C (Fig. 5 legend) and fractions of 1.0 ml were collected. *a*, Displays by SDS gel electrophoresis the pattern of polypeptides in 300- μ l samples from every other fraction from the gradient. *b*, Displays the pattern of hybridisation to Southern strips of RNA synthesised *in vitro* by 5- μ l samples from every fourth fraction from the gradient. *c*, Displays by polyacrylamide gel electrophoresis the *in vitro* synthesis of 120-base run-off RNA from 5- μ l samples from every fourth fraction from the gradient. Procedures for the experiments of *a*, *b* and *c* are as described for the corresponding panels of Fig. 5.

Initiation of spore development

We have isolated a form of RNA polymerase that transcribes a cloned gene³ (the 0.4-kilobase gene) that is activated at an early stage of sporulation in *B. subtilis*. As evidence of specific transcription, this RNA polymerase copied RNA selectively and asymmetrically from the 0.4-kilobase gene, initiating at a unique site close to the apparent startpoint for RNA synthesis *in vivo*. Nucleotide sequencing should now enable us to define this site precisely.

Purified RNA polymerase capable of transcribing the 0.4-kilobase gene was associated with a polypeptide of MW 37,000, termed P³⁷. P³⁷ co-purified with the 0.4-kilobase gene transcribing activity during gradient elution from both phosphocellulose and DNA-cellulose. It therefore seems likely that this species confers on RNA polymerase the ability to recognise and transcribe the 0.4-kilobase gene. This possibility can now be tested directly by reconstructing the enzyme complex from purified P³⁷ and core RNA polymerase. If P³⁷ is, in fact, a transcriptional determinant, we would suppose that, acting in the apparent absence of σ factor, it is itself a σ -like regulatory subunit of polymerase that directs recognition of the 0.4-kilobase gene promoter. As a precedent, a regulatory subunit of RNA polymerase encoded by *B. subtilis* phase SP01 causes core RNA polymerase to bind at the promoters for phase SP01 'middle' genes⁶.

The initiation of sporulation in *B. subtilis* is controlled by at least five or six genetic loci known as the stage 0 genes¹.

Mutations in stage 0 genes *spo0A*, *spo0B*, *spo0E*, *spo0F* and *spo0H* block sporulation at its earliest morphological stage (stage 0) and severely restrict transcription of the 0.4-kilobase gene *in vivo* (ref. 3 and J. F. Ollington, W.G.H. and R.L., in preparation). Based on these and other observations⁷⁻⁹, we believe that the stage 0 gene products turn on early sporulation genes by modifying RNA polymerase. It is tempting to suppose that P³⁷ is involved in this and that P³⁷ is itself the product of a stage 0 gene. In recent experiments, however, we find that P³⁷ is present in vegetative cells as well as in sporulating bacteria. Possibly, P³⁷ directs the transcription of several classes of genes but its involvement in sporulation RNA synthesis is specifically controlled by the stage 0 gene products. This could be investigated by identifying the structural gene for the P³⁷ protein.

The isolation from sporulating cells of an RNA polymerase which transcribed a sporulation gene *in vitro* is consistent with suggestions that spore development in general¹⁰ and the 0.4-kilobase gene in particular³ are controlled, at least in part, by one or more regulatory proteins that bind to RNA polymerase. It will now be of interest to determine whether temporally controlled classes of sporulation genes are transcribed by different forms of RNA polymerase containing distinct regulatory subunits.

We thank members of the Losick-Pero group who contributed reagents and advice and J. Pero and A. L. Sonenshein for assistance with the manuscript. This work was supported by NIH research grant 5 RO1 GM 18568 to R.L. and NIH postdoctoral fellowship 5 F32 GM 06401 to W.G.H.

Received 4 May; accepted 3 July 1979.

- Hoch, J. A. in *Advances in Genetics* Vol. 18 (ed. Caspari, E. W.) 67-98 (Academic, New York, 1976).
- Doi, R. A. *Rev. Genet.* **11**, 29-48 (1977).
- Segall, J. & Losick, R. *Cell* **11**, 751-761 (1977).
- Trowsdale, J., Chen, S. M. H. & Hoch, J. A. *Molec. gen. Genet.* **173**, 61-70 (1979).
- Southern, E. J. *molec. Biol.* **98**, 503-517 (1975).
- Talkingington, C. & Pero, J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1185-1189 (1978); *Virology* **83**, 365-379 (1977).
- Tjian, R. & Losick, R. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2872-2876 (1974).

- Tjian, R., Stinchcomb, D. & Losick, R. *J. biol. Chem.* **250**, 8824-8828 (1975).
- Segall, J., Tjian, R., Pero, J. & Losick, R. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4860-4863 (1974).
- Losick, R. & Sonenshein, A. L. *Nature* **224**, 35-37 (1969).
- Polisky, B. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 3310-3314 (1975).
- Shorenstein, R. & Losick, R. *J. biol. Chem.* **248**, 6163-6169 (1973).
- Denhardt, D. T. *Biochem. biophys. Res. Commun.* **23**, 641-646 (1966).
- Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-251 (1977).
- Hayward, G. S. *Virology* **49**, 342-344 (1972).
- Schaeffer, P., Millet, J. & Aubert, J. *Proc. natn. Acad. Sci. U.S.A.* **54**, 704-711 (1965).
- Studier, F. W. *J. molec. Biol.* **79**, 237-248 (1973).

Identification and processing of proglucagon in pancreatic islets

C. Patzelt, H. S. Tager, R. J. Carroll & D. F. Steiner

Department of Biochemistry, The University of Chicago, 920 East 58th Street, Chicago, Illinois 60637

Immunoprecipitation and tryptic peptide analysis of newly synthesised proteins from rat islets have identified an 18,000 molecular weight (MW) protein as proglucagon. Conversion of this precursor was kinetically similar to the conversion of proinsulin and resulted in the formation of both pancreatic glucagon and a 10,000-MW protein lacking this hormonal sequence.

AFTER the detection of proinsulin in 1967 (ref. 1) the search for a biosynthetic precursor of glucagon became the subject of considerable investigation. Several investigators have found larger immunoreactive forms of glucagon in extracts of pancreas^{2,3}, of gastric and intestinal mucosa^{4,5}, of salivary glands⁶, and in plasma⁷. Estimates of the molecular weights of these various forms range from 4,500 to 70,000, but only a few forms have been characterised. Thus, we isolated a peptide which carried a C-terminal extension of eight amino acids on the

sequence of pancreatic glucagon⁸. Because of its small size (MW 4,500) and because of the identification of low amounts of larger forms, this peptide seemed to represent a fragment or intermediate cleavage form of proglucagon. More recently, a larger form has been isolated from porcine intestine and has been partially sequenced. This peptide, glicentin⁹, consists of 100 amino acid residues and has a molecular weight of 12,000. The glucagon moiety in this molecule is also extended at the C-terminus by a sequence of eight amino acids very similar to that of the 4,500-MW proglucagon fragment. More recently, 12,000 and 8,000-MW glucagon-containing peptides of intestine have been found to have identical counterparts in pancreatic tissue¹⁰.

Although the above findings have not established the precursor nature of any of the larger forms of glucagon, biosynthetic studies in isolated fish islets have indicated that proglucagon has a molecular weight of 12,000 (refs 11, 12). In the present study we have used the high resolving power of SDS-polyacrylamide gel-electrophoresis and the sensitivity of fluorographic methods to examine the biosynthesis of glucagon in pancreatic islets of

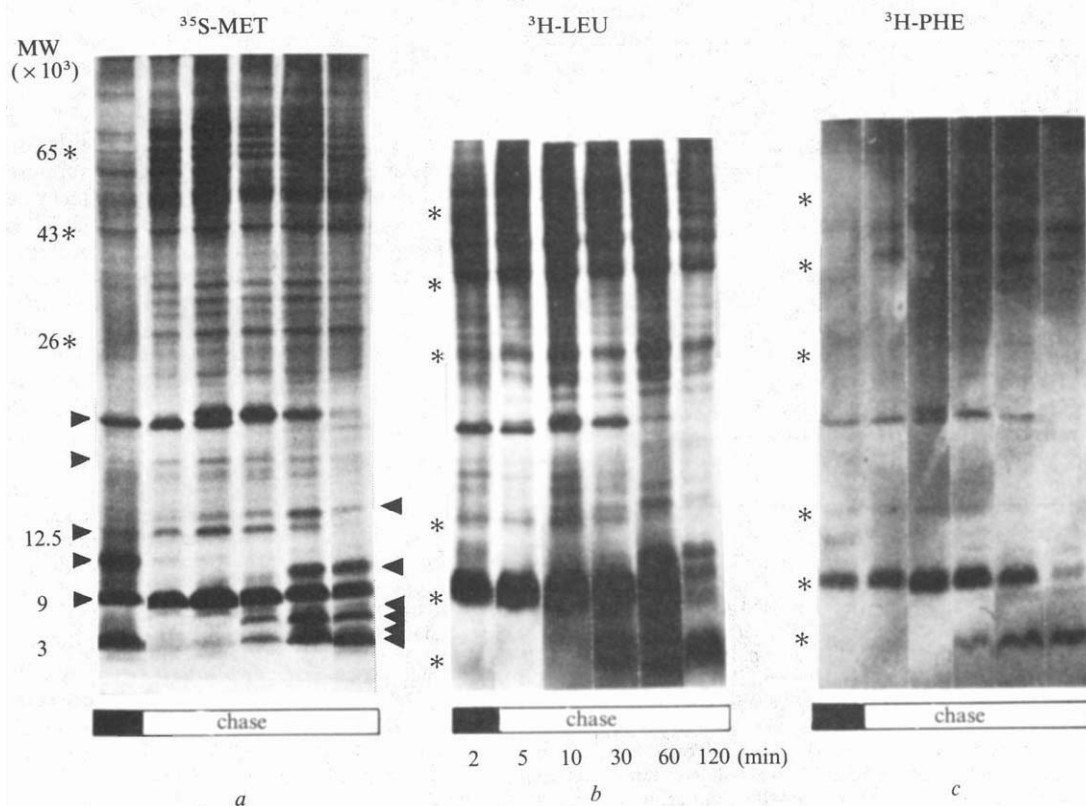


Fig. 1 Pulse-chase labeling of islet proteins. Samples of 100 isolated rat islets³⁰ were incubated in 50 μ l Hanks' buffer containing 2.5 mM glucose and 50 μ Ci of the indicated radioactive amino acid for 2 min at 37 °C. Pulse-labelling was stopped by the addition of 5 ml Hanks' buffer supplemented with 10^{-3} M of the respective cold amino acid. Chase periods, in minutes, were 0 (lane 1), 3 (lane 2), 8 (lane 3), 28 (lane 4), 58 (lane 5) and 118 (lane 6). Chase-incubation was stopped by chilling, centrifugation and boiling of islets in 12.5 μ l denaturing solution²⁰. Samples were electrophoresed on SDS-polyacrylamide gels (0.1% SDS/16% acrylamide/0.25% bisacrylamide^{16,31}) and dried gels were fluorographed^{32,33}. Asterisks show the position of marker proteins whose sizes are indicated numerically on the left. Arrowheads to the left of a point (from top to

bottom) to proglucagon (MW 18,000), an unidentified prohormone-like protein (MW 16,000), prosomatostatin (MW 12,500) (unpublished), preproinsulin¹⁶, and proinsulin. Arrowheads at the right point to conversion products.

the rat. Immunoprecipitation, tryptic peptide analysis and kinetic studies of formation and processing have all identified an 18,000-MW peptide as pancreatic proglucagon.

Prohormone-like proteins in pancreatic islets

To identify proteins having prohormone-like kinetics of formation and processing¹³⁻¹⁵, pulse-chase experiments were carried out with isolated rat islets using various radioactive amino acids of high specific activity. The slab gel electrophoretic system was optimised by adjusting the concentrations of acrylamide and bisacrylamide¹⁶ for resolution of proteins in the molecular weight range of 10,000–30,000, a size range in which new prohormones might be expected to occur¹⁷. In the pulse-chase experiments shown in Fig. 1 proinsulin was the predominant labelled band and was used as an internal standard for the detection of other proteolytically processed proteins. The most conspicuous protein which exhibited prohormone-like behaviour was a relatively highly labelled band of MW about 18,000, which appeared immediately after a pulse of 2 min. However, unlike proinsulin, this 18,000-MW protein underwent a small post-translational increase in its molecular size. It later disappeared after a chase period of 30–120 min. This period for post-translational processing is consistent with prohormone conversion, as indicated by the fading of the labelled proinsulin band and the appearance of smaller insulin-like conversion products (Fig. 1). These results suggested that the 18,000-MW band might represent a precursor to one of the other major islet hormones such as glucagon or somatostatin. Faintly labelled proteins showing similar labelling kinetics, though without early post-translational enlargement, could also be found consistently with MW 12,500 and, in the case of experiments *a* and *b* of Fig. 1, with 16,000 MW.

Proteins appearing later in the course of a chase-period are likely to represent intermediates or endproducts of the conversion process. Thus a 13,000-MW protein and one of MW 10,000 are, on the basis of their relative abundance, both

likely to be derived from the 18,000-MW precursor (Fig. 1). Furthermore, up to four labelled peptides smaller than proinsulin were resolved as indicated by arrowheads in Fig. 1a. These proteins were included, together with the above-mentioned candidate prohormones, in the surveys for proteins involved in the biosynthesis of glucagon described below. The 12,000-MW protein was identified as the prohormonal form of somatostatin (unpublished), whereas the 16,000-MW band has not yet been related to any known islet hormone.

Identification of glucagon-related proteins

For the identification of proglucagon and its conversion products islets were pulse-labelled for 2 h with ³⁵S-methionine. Islet lysates were electrophoresed and fluorographed as described in Fig. 1 legend and prohormones and conversion products were eluted from the dried gels for immunoprecipitation. Two types of glucagon-antisera were used for this study: the first is a tissue nonspecific antiserum (designated NS) known to be directed toward the central and N-terminal regions of glucagon and known to be reactive with larger forms of glucagon; the second is an antiserum specific for pancreatic glucagon (designated CTS, that is C-terminus-specific) which recognises the amino acid sequence at positions 24–29 of the glucagon molecule but which does not react with forms carrying a C-terminal extension in the radioimmunoassay¹⁰. Such an extension has been reported to be present in a proglucagon fragment isolated from commercial glucagon preparations⁸, in glicentin⁹, and in 12,000 and 8,000-MW glucagon-containing peptides of both pancreas and intestine¹⁰. It can be removed by combined treatment with trypsin and carboxypeptidase B. The authentic C-terminal tryptic fragment of glucagon cleaved out of a larger molecule by this enzymatic treatment would also be recognised by this specific antibody¹⁰.

The data in Fig. 2 shows that the two proteins of MW of 18,000 are the only candidate prohormones which exhibited significant reactivity with the glucagon antisera. In addition, a

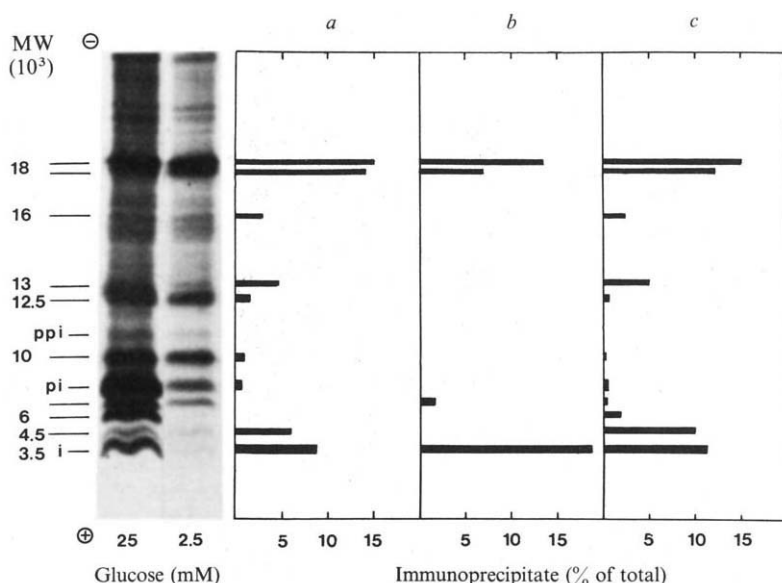


Fig. 2 Immunoprecipitation of newly synthesised islet proteins with various glucagon antisera. Islets (1,200) were incubated in Hanks' buffer containing 2.5 mM glucose and 600 μCi ^{35}S -methionine (Amersham) for 2 h at 37 °C. Islets were dissolved in 150 μl denaturing solution with boiling, the sample then being distributed to 12 slots of an SDS-polyacrylamide-gel^{7,16} slab for electrophoresis and subsequent fluorography. The lower molecular weight range of one lane of the resulting fluorograph is shown at the left side in comparison to a sample incubated at 25 mM glucose. Protein bands

as indicated at the margin (see also Fig. 1a) were eluted from excised gel pieces, either by shaking for 48 h at 37 °C in 2 $\times$ 2 ml of 0.01 M NH_4HCO_3 (proteins < 6,000-MW), or by shaking in 2 $\times$ 2 ml 25 mM Tris/200 mM glycine/0.1% SDS (proteins > 6,000-MW). Bovine serum albumin (BSA) (200 μg) was added to all samples as carrier. SDS was removed by dialysis against the SDS-free buffer for 72 h. Concentration of the samples was achieved by precipitation in 10% TCA or by lyophilisation (fractions < 6,000-MW). All fractions were finally dissolved in 0.7 ml NH_4HCO_3 (0.1 M). For immunoprecipitation 50- μl samples were incubated after addition of 150 μl of 0.1 M Tris-HCl, pH 7.6, 0.05 M NaCl, 0.01 M EDTA, 0.1% BSA with 1 μl glucagon antiserum NS (a) or CTS (b). Samples for set c were pretreated at analogous amounts with 2 μg tosylphenylalanine chloromethyl ketone-treated trypsin (Worthington) for 12 h at 37 °C in 0.5 ml 0.1 M NH_4HCO_3 /2 mM CaCl_2 . Reaction was stopped by addition of 5 μg phenylmethylsulphonyl fluoride (in absolute ethanol) and 10 μg carboxypeptidase B (Boehringer) was added for a further incubation period of 12 h at 37 °C. Reaction was finally stopped by boiling, samples were lyophilised and prepared for immunoprecipitation with antibody CTS as described above. Controls to which 10 μg of glucagon were added before the addition of the antiserum were always included. All samples were run as duplicates. After 24 h incubation the appropriate anti-IgG serum was added for double-antibody precipitation. Precipitates were washed in excess buffer containing 1% Triton X-100. Pellets were finally dissolved in 0.2 ml 3 M acetic acid and counted in 10 ml Aquasol (New England Nuclear). Data represent means of two different experiments (except for the 13,000 and 16,000-MW proteins). ppi, Preproinsulin; pi, proinsulin; i, insulin.

low molecular weight fraction co-migrating with iodinated glucagon (MW 3,500) and a somewhat slower migrating peptide (MW 4,500) exhibited immunoreactivity; to a lesser extent the 13,000-MW protein was also precipitated with antiglucagon serum. Whereas the 3,500-MW fraction reacted with the C-terminus specific antibody without enzymatic pretreatment (Fig. 2b) the 4,500 and 13,000-MW proteins showed such reactivity only after trypsin plus carboxypeptidase B-treatment (Fig. 2b versus c) and may thus carry a C-terminal extension on their glucagon moieties. On the basis of its estimated size and its immunological behaviour, the 3,500-MW peptide is regarded as intact glucagon, whereas the 4,500-MW glucagon-like component probably represents the proglucagon fragment described earlier⁸. The 13,000-MW protein, which might be contaminated by the adjacent band (prosomatostatin), may be an intermediate in the conversion process. Immunoprecipitation of the intact 18,000-MW peptides by the C-terminus-specific antibody was not unexpected, inasmuch as this antiserum will react with C-extended forms in conditions of antibody excess, presumably due to the presence of small amounts of non-discriminating antibodies (unpublished results).

To clarify the nature of the 18,000-MW component the labelled 18,000-MW protein was subjected to tryptic cleavage and two-dimensional analysis. The presence of the labelled peptides of glucagon in the tryptic digest would confirm the identification of this protein as proglucagon. Analysis of the C-terminal tryptic fragment(s) before and after treatment with carboxypeptidase B would allow the detection of an additional C-terminal basic amino acid, indicating the presence of an extension at this site. The 10,000-MW fragment which, as shown in Fig. 2, was found to be unreactive with the glucagon antisera, was included in this peptide analysis. The relevant peptide maps are presented in Fig. 3. It is evident from these

maps that the 18,000-MW protein contained the N-terminal tryptic peptide (labelled with ^3H -phenylalanine, Fig. 3c, left panel) as well as the group of four peptides related to the C-terminal tryptic fragment of glucagon. This latter group of peptides, however, migrated to the positions of the respective ninhydrin-stained peptides of the glucagon carrier only if the tryptic digestion products were treated with carboxypeptidase B (Fig. 3b, left panel). Trypsin-treatment alone did not result in the formation of these ^{35}S -methionine-(Fig. 3a) or ^3H -phenylalanine-(Fig. 3c) labelled peptides of the glucagon-C-terminus, thus supporting the assumption that the C-terminus of the glucagon-moiety in this prohormone is extended by an additional sequence.

In addition, release of the N-terminal tryptic peptide of glucagon during digestion of the 18,000-MW protein with trypsin (Fig. 3c, left panel) shows that the glucagon sequence in this precursor is linked to its N-terminal extension by at least one basic residue.

Comparison of the maps of the 18,000-MW protein with those of the 10,000-MW protein (Fig. 3, left versus right panels, respectively) shows that these two proteins have many tryptic peptides in common, but that digests of the 10,000-MW fragment lack the corresponding glucagon-related peptides. These results agree with the immunoprecipitation data of Fig. 2, that is, the glucagon-related sequence was removed during the conversion of proglucagon to the 10,000-MW fragment.

Conversion of proglucagon

These results suggest that the breakdown of the 18,000-MW proglucagon to its 10,000-MW fragment should be accompanied either by the generation of a glucagon-containing fragment or by the formation of labelled glucagon itself. For a

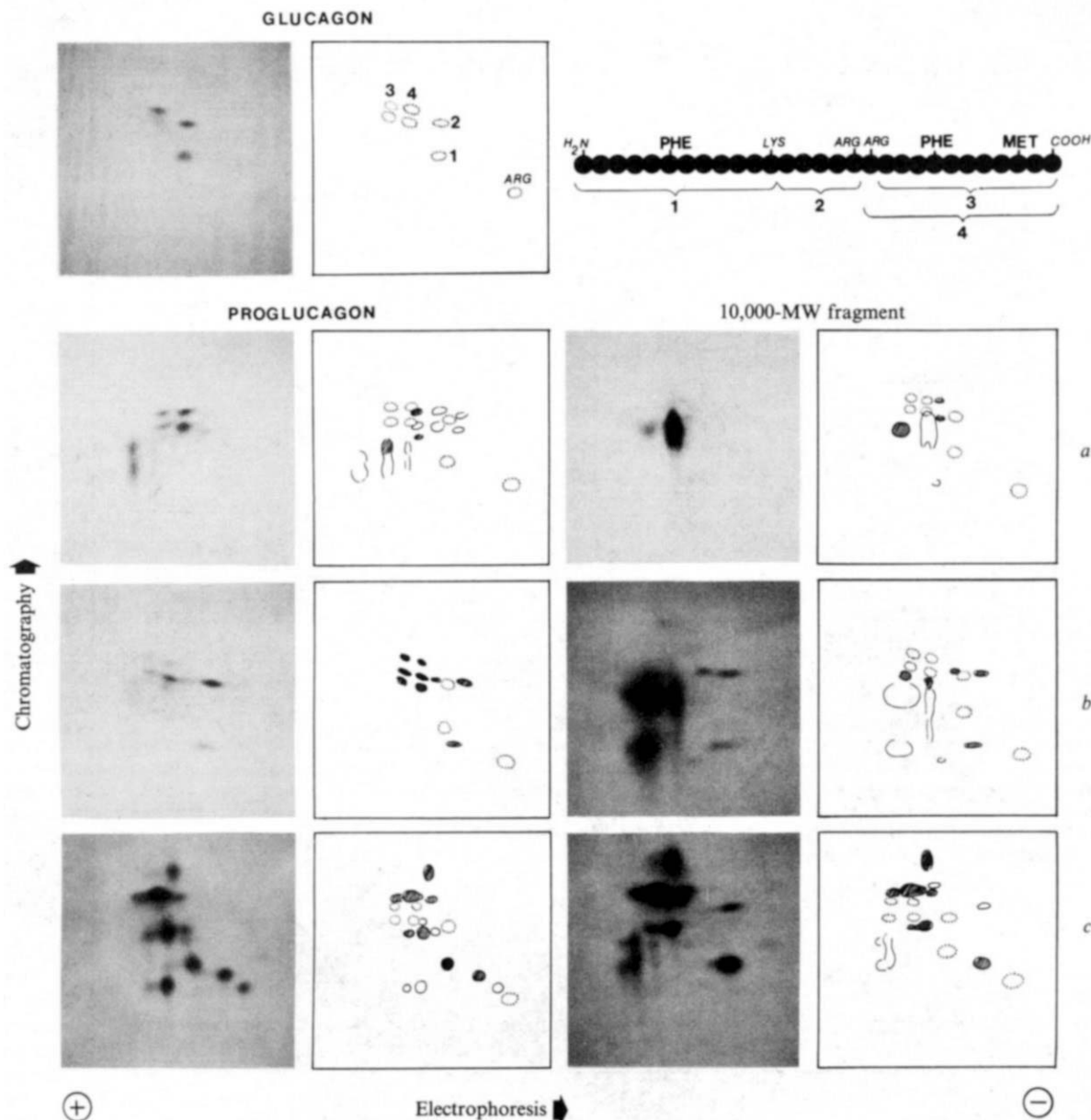


Fig. 3 Tryptic peptide analysis of proglucagon and its 10,000-MW fragment. 18,000 and 10,000-MW proteins, labelled for 2 h and eluted from the fluorographed electrophoresis gel, were treated with trypsin (*a* and *c*) or trypsin plus carboxypeptidase B (*b*) as described in Fig. 2 legend. The proteolytic digests were lyophilised, the tryptic peptides of 50 µg glucagon were added, and samples were electrophoresed on 20 × 20 cm cellulose-thin layer plates in 30% formic acid in the first and chromatographed in *n*-butanol/pyridine/acetic acid/water (60:40:12:48, v/v) in the second dimension. Plates were coated in PPO³⁴ and fluorographed. After exposure, plates were stained with ninhydrin reagent for detection of the peptides of the glucagon carrier. The figure shows at the upper left the pattern of the tryptic fragments of glucagon. Set *a* shows the fluorographed peptide maps of the tryptic digests of the respective ³⁵S-methionine labelled proteins, set *b* the trypsin + carboxypeptidase B digests of the same proteins, whereas under *c* the tryptic peptides of the ³H-phenylalanine-labelled proteins are shown. Diagrams at the right side of the respective maps: dotted spots, ninhydrin-stained glucagon peptides (not visible on the fluorographs); hatched spots, peptides that are common to both proteins (proglucagon and 10,000-MW fragment); black spots, labelled spots on the fluorograph at the position of ninhydrin-stained tryptic glucagon fragment(s).

comparison of the kinetics of formation of proglucagon and its fragments, islets were 'pulse'- and 'pulse-chase'-labelled. The indicated bands were excised from the fluorographed gels and the radioactivity was quantitated by liquid scintillation spectrometry. Due to the similarity of the peptide patterns of proglucagon and the 10,000-MW fragment (Fig. 3) the presence of major contaminants in these bands is unlikely. Because of contaminating labelled peptides in the 3,500-MW band, labelled glucagon was quantitated after immunoprecipitation of the eluted material.

The data of the pulse-chase experiment illustrated in Fig. 4a show that the prohormone was converted into its slightly larger form within 10–30 min, and then began to decrease as label appeared in the 10,000-MW component. Immunoprecipitable,

labelled glucagon was not detected in this experiment, however. With prolonged pulse-labelling (Fig. 4b) the expected saturation of incorporation of labelled amino acids into the two forms of proglucagon occurred within the first 60 min. Formation of the 10,000-MW protein began after a delay of 30–60 min and then continued almost linearly for the period up to 3 h. Labelled glucagon was detected in the eluate of the 3,500-MW band (Fig. 4b bottom panel), but its formation seemed to be somewhat slower than that of the 10,000-MW fragment. The 13,000-MW protein which showed some glucagon-like immunoreactivity (Fig. 2) was also included in these analyses. However, its kinetic behaviour did not clearly indicate whether this protein is a transient intermediate or a side-product of the conversion process (see discussion).

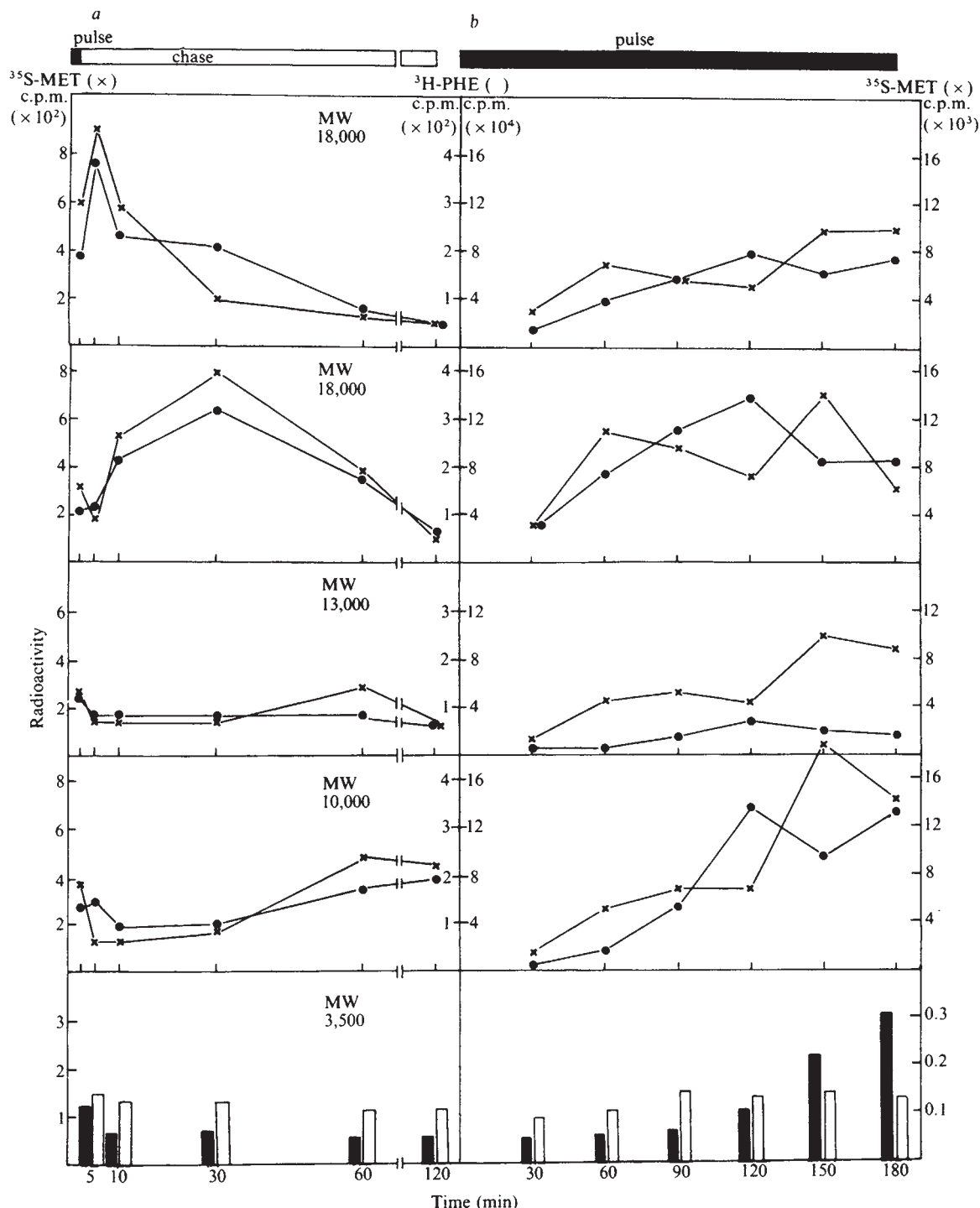


Fig. 4 Kinetics of formation and conversion of glucagon-related proteins. *a*, The pulse-chase experiments are those shown in Fig. 1*a* and *c* (see legend for details). *b*, For prolonged pulse-labelling samples of 100 islets were incubated for the indicated period and islet lysates were treated for electrophoresis as described before. The respective bands were excised from fluorographed gels, dissolved in 0.2 ml 30% H_2O_2 and counted in 10 ml Aquasol. The 3,500-MW protein was eluted from the respective band and immunoprecipitated as described in Fig. 2 legend. All data represent means of two determinations. ■, Immunoprecipitates; □, controls.

Comparison of the scales in Fig. 4 shows that the accumulation of label in the 10,000-MW fragment and in glucagon, respectively, differ by a factor of about 50. This discrepancy may be due to the presence of more than one methionine residue in the 10,000-MW protein, incomplete immunoprecipitation of glucagon or the accumulation of other glucagon-containing intermediates similar to the 4,500-MW peptide (Fig. 2) which were not included in these calculations. Furthermore, considerable losses of glucagon from the gel may have occurred during treatment for fluorography. Such losses have been found to be

negligible for proteins in the range larger than 8,000–9,000 MW.

Quantitative aspects of prohormone synthesis in pancreatic islets

Pancreatic islets are composed of at least four different cell types each of which produces at least one known hormone, namely B cells (insulin), A cells (glucagon), D cells (somatostatin) and a fourth cell type which produces pancreatic polypeptide. The relative numbers of these cell types have been determined in

Table 1 Comparison of cellular composition and prohormone biosynthesis in pancreatic rat islets

Cellular composition			Prohormone synthesis			
Cell type	Hormone	% Of islet volume	Prohormone	MW	¹⁴ C-AA incorporation(c.p.m.)*	
					a	b
B cell	Insulin	56	Proinsulin	9,000	4,561 (66)	15,520 (87)
A cell	Glucagon	16	Proglucagon	18,000	1,766 (26)	1,566 (8)
D cell	Somatostatin	5	Prosomatostatin	12,500	200 (3)	287 (2)
PP cell	Pancreatic polypeptide	5	Pancreatic propolypeptide	—		
?	?		Unidentified prohormone	16,000	307 (5)	410 (3)

* Samples of 100 islets were preincubated for 2 h at 37 °C at 2.5 (a) or 25 (b) mM glucose. 20 μ Ci of a ¹⁴C-labelled amino acid mixture (Amersham) was then added and incubation was continued for 20 min. Islets were washed, lysed and electrophoresed as described in the Fig. 1 legend. The respective bands were excised from the fluorographed gels and scintillation-counted (see Fig. 4 legend). Data represent means of four determinations. Relative prohormone synthesis is given in parentheses.

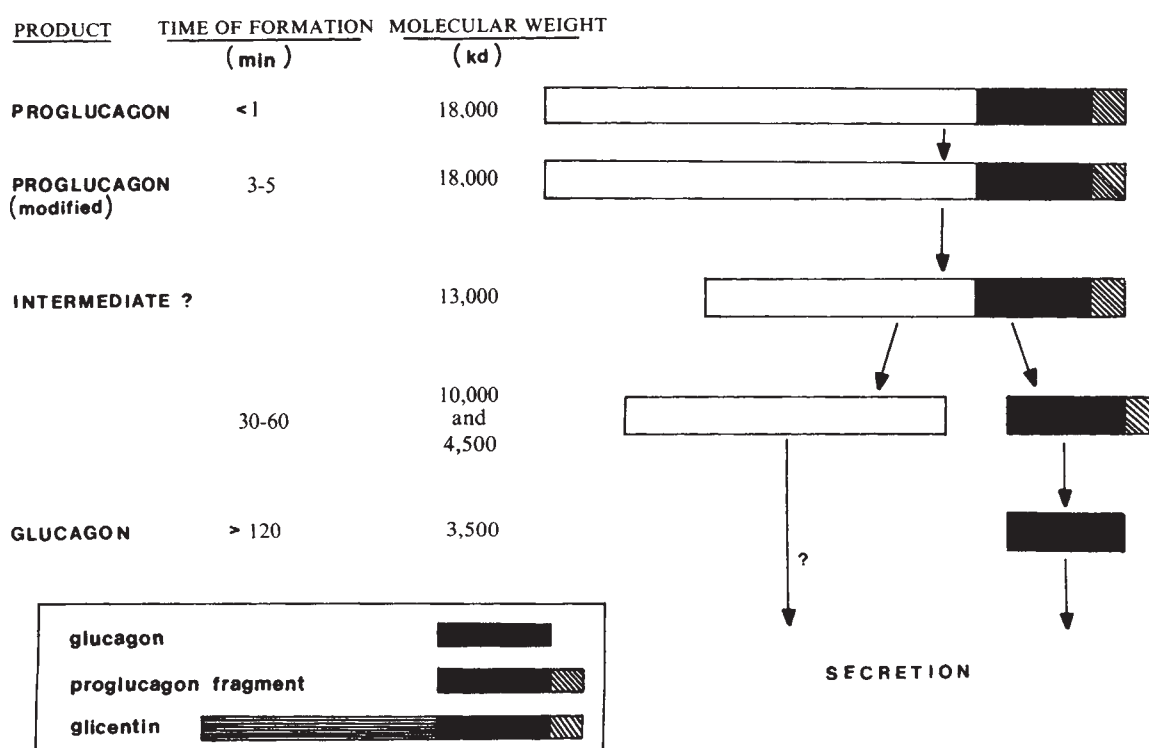
islets of several species, including rats^{18,19}. Table 1 gives a synopsis of these data in comparison with the individual rates of biosynthesis of the prohormones identified thus far. As glucose has been reported not only to have a stimulatory effect on insulin synthesis but also an inhibitory effect on the synthesis of glucagon^{20,21}, prohormone synthesis was studied after preincubation of islets for 2 h at low (2.5 mM) and high (25 mM) glucose concentrations. To compensate for differences in amino acid composition, incorporation was measured with a mixture of ¹⁴C-labelled amino acids. Although this experiment is based on some assumptions, the biosynthetic data compiled in Table 1 (right panel) correlate remarkably well with the proportion of the various cell types in rat islets (Table 1, left panel). Whereas proinsulin-synthesis was stimulated fourfold by preincubation at high glucose concentrations the formation of the other prohormones remained almost unaltered in agreement with our own previous findings (Fig. 2, ref. 11).

Discussion

On the basis of its kinetics of formation and processing, immunological analysis and tryptic peptide mapping, the

18,000-MW protein of rat pancreatic islets must be regarded as proglucagon. Final proof of this conclusion may come from the identification of the primary translation product of the respective mRNA as preproglucagon, the putative presecretory form which, if typical, should have a molecular weight of about 21,000. Preliminary evidence for such a translation production has been obtained by translation of RNA extracted from a human pancreatic endocrine tumour³⁵. Although preproinsulin was detectable after pulse labelling of intact islets (Fig. 1, ref. 11), a similarly short-lived protein of the presumed size of preproglucagon was not detectable in the experiments reported here. The post translational modification of the 18,000-MW protein which results in an increase in its molecular size has not yet been identified. Glycosylation does not seem to be involved as judged from the very low incorporation of various labelled sugars, the lack of binding to various lectins, and the lack of any inhibition of its biosynthesis by tunicamycin, a known inhibitor of protein glycosylation²².

With regard to the position of the glucagon sequence within the prohormone, we can conclude that the hormonal sequence is not located at the C-terminus of the precursor. The arrangement

**Fig. 5** Proposed scheme for the conversion of proglucagon to glucagon in pancreatic rat islets.

proposed in the scheme shown in Fig. 5 was chosen by analogy to the position of glucagon in glicentin⁹, which has been demonstrated by immunohistochemistry to be present also in the rat pancreatic A cell²³. Immunoprecipitation of the proteins listed in Fig. 2 with antiserum R-64, which is directed towards the non-glucagon portion of porcine glicentin²⁴, however, did not provide evidence for such immunoreactivity in proglucagon or any of its conversion products. This lack of reactivity may most simply be interpreted to indicate that larger forms of glicentin are poorly reactive with this antiserum or that some damage to the antigenic site has occurred during electrophoresis and elution of the peptides. Alternatively, it is possible that multiple genes for proglucagon-like peptides occur in the rat as is known to be the case with the insulin gene¹², and that glicentin is a minor component of the pancreatic alpha cell¹⁰.

The sequential conversion of proglucagon can only be inferred to correspond to the scheme shown in Fig. 5. Our data support the conclusion that, in addition to glucagon, the 10,000-MW fragment is an end product of proglucagon conversion and might therefore represent a so far unidentified hormonal entity. It is not yet known whether this fragment is secreted along with glucagon. The 13,000 and 4,500-MW may represent intermediates in the conversion process, but they could also represent side-products of this process. Similar explanations

may apply to several larger forms detected in glucagon-producing tumours²⁵, or in plasma in normal and various clinical conditions^{7,25}. In any case, such intermediates or side products may have escaped detection during the 2-h period used in our biosynthetic experiments, whereas the short-lived prohormone might be overlooked during immunochemical studies on tissue extracts.

This prohormone, about five times the size of glucagon, might well contain additional hormonal sequences. Immunohistochemical techniques have suggested that additional hormones, including gastric inhibitory polypeptide^{26,27}, cholecystokinin²⁸ and even β -endorphin²⁹, may be present in pancreatic A cells. Our identification of a distinct protein as the prohormone of glucagon represents the first step in its further characterisation, which will ultimately answer the question of the possible presence of additional hormonal sequences, the question of the nature of the post translational modification as well as that on the mode of prohormone conversion.

We thank Nora Walter for help in manuscript preparation and Dr A. J. Moody, New Research Institute for R-64 antiserum. This work was supported by research grants AM 13914, AM 18347 and AM 20595 from the US PHS and NIH and by a grant from the Kroc Foundation. H.S.T. is the recipient of Research Cancer Development Award AM 00145 from the NIH.

Received 29 May 1979; accepted 3 September 1979.

- Steiner, D. F., Cunningham, D., Spiegelman, L. & Aten, B. *Science* **157**, 697-700 (1967).
- Rigopoulou, D., Valverde, I., Marco, J., Faloona, G. R. & Unger, R. H. *J. biol. Chem.* **245**, 496-501 (1970).
- Hellerstrom, C., Howell, S. L., Edwards, J. C. & Andersson, A. *FEBS Lett.* **27**, 97-101 (1972).
- Valverde, I., Rigopoulou, D., Marco, J., Faloona, G. R. & Unger, R. H. *Diabetes* **19**, 614-623 (1970).
- Srikant, C. B., McCorkle, K. & Unger, R. H. *Metabolism* **25** (suppl. 1), 1403-1404 (1976).
- Lawrence, A. M., Tan, S., Hojvat, S. & Kirsteins, L. *Science* **195**, 70-72 (1976).
- Valverde, I., Villaneuva, M. L., Lozano, I. & Marco, J. *J. clin. endocr. Metab.* **39**, 1020-1028 (1974).
- Tager, H. S. & Steiner, D. F. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2321-2324 (1973).
- Jacobson, H., Demandt, A., Moody, A. J. & Sundby, F. *Biochim. biophys. Acta* **493**, 452-459 (1977).
- Tager, H. S. & Markese, J. *J. biol. Chem.* **254**, 2229-2233 (1979).
- Noe, B. D. & Bauer, G. E. *Endocrinology* **89**, 624-651 (1971).
- Noe, B. D. & Bauer, G. E. *Endocrinology* **97**, 868-877 (1975).
- Steiner, D. F., Kemmler, W., Clark, J. L., Oyer, P. E. & Rubenstein, A. H. in *Handbook of Physiology-Endocrinology I* (eds. Steiner, D. F. & Freinkel, N.) 175-198 (Williams and Wilkins, Baltimore, Maryland, 1972).
- Cohn, D. V., Macgregor, R. R., Chu, L. L. H., Kimmel, J. R. & Hamilton, J. W. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1521-1525 (1972).
- Roberts, J. L., Phillips, M., Rosa, P. A. & Herbert, E. *Biochemistry* **17**, 3609-3618 (1978).

- Patzelt, C. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 1260-1264 (1978).
- Patzelt, C. *et al. Proc. 11th FEBS Meet. Copenhagen*, **47**, Sym A6, 69-78 (Pergamon, Oxford and New York, 1978).
- Orci, L. *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 1338-1342 (1976).
- Sundler, R., Håkanson, R., Lundquist, I. & Larsson, L.-I. *Cell Tissue Res.* **178**, 307-312 (1977).
- Tung, A. K. & Zeregu, F. *Biochem. biophys. Res. Commun.* **45**, 387-395 (1971).
- O'Connor, K. J., Gay, A. & Lazarus, N. R. *Biochem. J.* **134**, 473-480 (1973).
- Takatsuki, A. & Tamura, G. *J. Antibio. (Tokyo) Ser. A*, **24**, 785-794 (1971).
- Moody, A. J. *et al. in Glucagon, Its Role in Physiology and Clinical Medicine* (eds Foa, P. P., Bajaj, J. S. & Foa, N. L.) 129-138 (Excerpta Medica, Amsterdam, 1977).
- Moody, A. J., Jacobsen, H. & Sundby, F. in *Gut Hormones* (ed. Bloom, S. R.) 369-378 (Churchill & Livingstone, Edinburgh, 1978).
- Jaspan, J. B. *et al. Metabolism* **25** (suppl. 1), 1397-1401 (1976).
- Smith, P. H., Merchant, F. W., Johnson, D. G., Fujimoto, W. Y. & Williams, R. H. *Analyt. Rec.* **149**, 585-590 (1977).
- Alumets, J., Håkanson, R., O'Dorisio, T., Sjölund, K. & Sundler, F. *Histochemistry* **58**, 253-257 (1978).
- Grube, D., Maier, V., Raptis, S. & Schlegel, W. *Histochemistry* **56**, 13-35 (1979).
- Grube, D., Voigt, K. H. & Weber, E. *Histochemistry* **59**, 75-79 (1978).
- Lenmark, Å., Nathans, A. & Steiner, D. F. *J. Cell Biol.* **71**, 606-623 (1976).
- Laemmli, U. K. *Nature new Biol.* **227**, 680-685 (1970).
- Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83-88 (1974).
- Laskey, R. A. & Mills, A. D. *Eur. J. Biochem.* **56**, 335-341 (1975).
- Randerath, K. *Analyt. Chem.* **41**, 991-992 (1969).
- Patzelt, C. *et al. Proc. 10th Congress Internat. Diabetes Fedn*, 1979 (Elsevier, in the press).

The heavy chain of human histocompatibility antigen HLA-B7 contains an immunoglobulin-like region

Harry T. Orr, Doron Lancet, Richard J. Robb, José A. Lopez de Castro & Jack L. Strominger

Harvard University, The Biological Laboratories, Cambridge, Massachusetts 02138

The 88-residue fragment (ac-2) containing the second disulphide loop from HLA-B7 heavy chain is shown to have statistically significant homology with Ig constant domains, including both matches at invariant positions and conservative substitutions of structurally important residues. Thus, both the light chain (β_2m) and a segment of the heavy chain of HLA antigens may be structurally and evolutionarily related to immunoglobulins.

AMONG the products encoded by the major histocompatibility complex (MHC)¹⁻³ are the highly polymorphic antigens coded for by the H-2K and H-2D loci in the mouse and the HLA-A, HLA-B and HLA-C loci in man. Besides being target antigens during graft rejection, these proteins are important in the recognition of both virally infected^{4,5} and chemically modified^{6,7} syngeneic cells by cytotoxic T lymphocytes. These membrane antigens consist of a glycosylated heavy chain of MW 44,000 and a light chain of MW 12,000⁸⁻¹¹. The larger chain is variant and is thought to be coded for by the MHC. The smaller chain is invariant and has been identified as β_2 -microglobulin (β_2m)

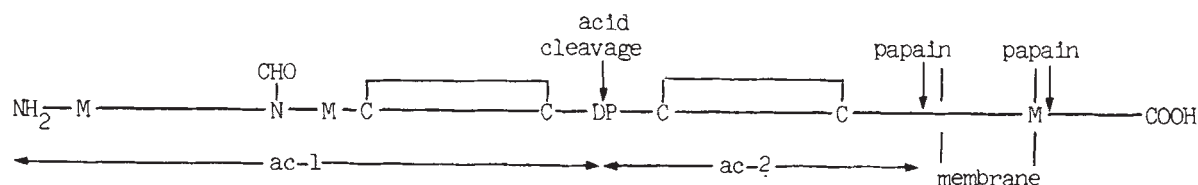


Fig. 1 Localisation of the ac-2 fragment within the HLA-B7 heavy chain. Papain-solubilised HLA-B7 heavy chain (MW 34,000) was purified from the human lymphoblastoid cell line JY as described elsewhere²¹. The ac-2 fragment was obtained by partial acid hydrolysis in 70% formic acid at 37 °C (ref. 19) followed by gel filtration. ac, acid cleavage fragment; CHO, carbohydrate moiety; M, Met; N, Asn; C, Cys; D, Asp; P, Pro.

which is not coded for by the MHC. An interesting feature of β_2 -m is its primary structural homology with immunoglobulin (Ig) constant domains^{12,13}. As the constituent chains of many multi-subunit proteins are structurally homologous (for example, haemoglobins, immunoglobulins and many multi-subunit enzymes), it was tempting to speculate that the heavy chain of HLA-A and -B would contain segments resembling β_2 m and therefore Ig-like domains. Such homology would also further support the notion of common evolutionary origins for the immunoglobulin and histocompatibility systems¹⁴⁻¹⁶. Early reports of HLA-A and -B similarity to Ig were based on indirect evidence such as overall subunit structure^{17,18}, susceptibility to limited papainolysis¹⁸ and the arrangement¹⁹ and size²⁰ of the intrachain disulphide loops. When preliminary data on the primary structure of the HLA-A and -B heavy chain became available, several authors reported sequence homologies with β_2 m and various Ig domains^{19,21-24}. However, all of these preliminary reports were based on short segments of the HLA-A and -B heavy chain.

In this article, the complete amino acid sequence of the 88-residue fragment (ac-2) containing the second disulphide loop of the HLA-B7 heavy chain is shown to display statistically significant homologies to Ig constant domains and β_2 m. This homology is discussed in terms of its possible relation to structure and evolution.

Comparison of the ac-2 fragment of HLA-B7 and Ig constant domains

The ac-2 fragment comprises the COOH-terminal region of papain-solubilised HLA-B7 heavy chain (see Fig. 1). Sequence determination of papain-solubilised HLA-B7 heavy chain, including ac-2, is described in detail elsewhere^{25,26}. The sequence of the ac-2 fragment was aligned with human Ig constant domains and β_2 m²⁷⁻³² (Fig. 2; see legend for detailed procedure). These latter proteins are members of a single superfamily³².

The significance of the sequence homology between ac-2 and Ig constant domains becomes more apparent by considering the positions involved: many of those are highly conserved among different Ig-like domains. The two cysteines (29 and 98) appear in all sequences of Fig. 2 and actually in all Ig domains, including the variable ones. Trp 45 is also very prominent, appearing in all Ig constant and variable domains. In the three-dimensional structure they are known to occupy a position at the centre of the domain, near the internal disulphide bridge, and probably have a role in chain folding (ref. 33 and refs cited therein). Notably, β_2 m does not have this tryptophan, which is replaced by Leu 45. Val 100 and His 102 appear in all but one, Pro 36 appears in all but two and Val 8 appears in all but three sequences in Fig. 2. Of seven residues marked as particularly conserved (that is, they

Table 1 Difference matrix

	C _L			C _{H1}	C _{H2}	C _{H3}				C _{H4}	β _{2m}	ac-2				
C _A	62	62		76	78	77				75	82	78	C _L			
C _{A1}	75	68		65	82	82				80	88	86	C _{H1}			
C _{μ1}	82	79	65													
C _{μ2}	76	79	79	85		78				79	85	79	C _{H2}			
C _{A3}	73	76	77	85	77	70				77	86	83	C _{H3}			
C _{μ3}	80	78	81	85	78									73		
C _{ε3}	79	77	79	86	80									67	70	
C _{A4}	75	72	73	82	79	76	76	76	68				81	81	C _{H4}	
C _{μ4}	78	72	76	84	78	72	73	70								64
C _{ε4}	80	74	81	86	81	82	86	79								68
β _{2m}	80	85	87	90	85	85	86	88	76	86	82	77	β _{2m}			
ac-2	78	79	82	89	79	87	80	82	79	81	83					
avg	76.5	75.1	76.9	83.1	79.6	77.5	78.8	77.8	74.7	75.6	79.5	83.9	81.3			
	C _κ	C _A	C _{γ1}	C _{μ1}	C _{μ2}	C _{γ3}	C _{μ3}	C _{ε3}	C _{γ4}	C _{μ4}	C _{ε4}	β _{2m}	ac-2			

The difference matrix²⁷ derived from the alignment of Fig. 2 is shown in the section below the diagonal line. Numbers represent per cent non-identity between pairs of sequences (including matches between a gap and any residue). All values except those involving ac-2 are reproduced from ref. 32. The numbers for ac-2 are calculated as upper limits, assuming that no identities occur beyond position 110. The numbers above the diagonal line are obtained by averaging the values of corresponding domains from different Ig chain classes. The numbers in the lowest row are averages of difference values between a particular sequence and all others. Values for a sequence with itself are excluded from all calculations.

Fig. 2

Human Ig C domains		10										20										30													
Light chains																																			
1 kappa		—	T	V	A	A	P	S	V	F	I	F	P	P	S	—	D	E	Q	L	K	—	S	G	T	A	S	V	V	C	L	22			
2 lambda		Q	P	K	A	A	P	S	V	T	L	F	P	P	S	—	S	E	E	L	Q	—	A	N	K	A	T	L	V	C	L	20			
Heavy chains																																			
3 C _γ 1		A	S	T	K	G	P	S	V	F	P	L	A	P	S	—	S	K	S	T	S	—	G	G	T	A	A	L	G	C	L	17			
4 C _γ 1		G	S	A	S	A	P	T	L	F	P	L	V	S	C	—	E	B	S	B	P	—	S	S	T	V	A	V	G	C	L	14			
5 C _γ 2		Z	L	P	P	K	V	S	V	F	V	—	P	P	R	—	B	G	F	F	G	B	P	R	K	T	V	K	L	I	C	Q	24		
6 C _γ 3		E	L	L	G	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P	E	V	T	C	V	15			
7 C _γ 3		D	Z	B	T	A	I	R	V	F	A	I	P	P	S	—	F	A	S	I	F	L	T	K	S	T	P	T	L	T	C	L	20		
8 C _γ 3		S	D	P	R	G	V	S	A	Y	L	S	R	P	S	P	F	D	—	L	F	I	R	K	S	P	T	K	L	T	C	L	19		
9 C _γ 4		Q	P	R	—	E	P	Q	V	Y	T	L	P	P	S	—	R	E	E	M	T	—	K	N	Q	V	S	L	T	C	L	19			
10 C _γ 4		V	A	L	H	R	P	D	V	Y	L	P	P	A	—	R	E	Q	L	N	L	R	E	S	A	T	I	T	C	L	22				
11 C _γ 4		—	P	R	A	A	P	E	V	Y	A	F	A	T	P	—	E	W	P	G	S	—	R	D	K	R	T	L	A	C	L	17			
HLA chains																																			
12 β _{2m}		I	Q	R	—	T	P	K	I	Q	V	Y	S	R	H	—	P	A	E	N	G	—	K	S	N	F	—	L	N	C	Y	19			
13 B7, ac-2		/	D	P	P	K	T	H	V	T	H	H	P	—	—	—	—	—	—	I	S	—	D	H	E	A	T	L	R	C	W				
(a)																																			
(b)																																			
Human Ig C domains		40										50										60													
Light chains																																			
1 kappa		L	N	N	F	Y	P	R	E	—	—	A	K	V	Q	W	K	V	—	—	—	D	N	A	L	Q	S	G	N	S	Q	—	22		
2 lambda		I	S	D	F	Y	P	G	A	—	—	V	T	V	A	W	K	A	—	—	—	D	S	S	P	V	K	A	G	V	—	—	20		
Heavy chains																																			
3 C _γ 1		V	K	D	Y	F	P	E	P	—	—	V	T	V	S	W	—	—	—	—	N	S	G	A	L	T	S	G	V	—	—	17			
4 C _γ 1		A	Q	D	F	L	P	D	S	—	—	I	T	F	S	W	K	Y	K	B	N	S	D	I	—	—	S	S	T	—	—	14			
5 C _γ 2		A	T	G	F	S	P	R	Q	—	—	I	Q	V	S	W	L	R	—	—	E	G	G	V	Q	V	H	N	—	—	A	24			
6 C _γ 3		V	V	D	V	S	H	E	D	P	Q	V	K	F	N	W	Y	V	—	—	D	G	K	Q	V	V	H	N	—	—	A	15			
7 C _γ 3		V	T	D	L	T	—	Y	D	—	—	S	V	T	I	S	W	T	R	—	—	Q	D	G	E	A	V	K	—	—	T	20			
8 C _γ 3		V	V	B	L	A	P	S	K	G	T	V	B	L	T	W	S	R	—	—	A	S	G	K	P	V	B	—	—	H	19				
9 C _γ 4		V	K	G	F	Y	P	S	D	—	—	I	A	V	E	W	E	S	—	—	N	D	G	E	P	F	N	—	—	Y	19				
10 C _γ 4		V	T	G	F	S	P	A	D	—	—	V	F	V	Q	W	Q	M	—	—	Q	R	G	Q	P	L	S	P	E	K	Y	22			
11 C _γ 4		I	Q	N	F	M	P	E	D	—	—	I	S	V	Q	W	L	H	—	—	N	E	V	Q	L	P	D	—	A	R	H	17			
HLA chains																																			
12 β _{2m}		V	S	G	F	H	P	S	D	—	—	I	E	V	D	L	L	K	—	—	D	G	E	R	I	E	K	—	—	—	V	19			
13 B7, ac-2		A	L	G	F	Y	P	A	E	—	—	I	T	L	T	W	Q	R	—	—	D	G	E	D	Q	T	Q	D	—	—	T				
(a)																																			
(b)																																			
Human Ig C domains		70										80										90													
Light chains																																			
1 kappa		E	S	V	T	E	Q	D	S	K	S	D	—	—	S	T	Y	S	L	S	S	T	L	T	L	S	K	A	D	Y	E	—	22		
2 lambda		E	T	T	T	P	S	K	Q	S	N	—	—	N	K	Y	A	A	S	S	Y	L	S	L	T	P	E	Q	W	K	—	20			
Heavy chains																																			
3 C _γ 1		H	T	F	P	A	V	L	Q	S	S	—	—	G	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	L	—	—	17			
4 C _γ 1		R	G	F	P	S	V	L	R	G	S	—	—	G	K	Y	A	A	T	S	Q	V	L	L	P	S	K	D	V	M	Q	14			
5 C _γ 2		N	E	V	Z	A	Z	A	K	E	S	G	P	T	T	Y	K	V	T	S	T	V	L	T	I	K	E	S	B	W	L	—	24		
6 C _γ 3		K	T	K	P	R	E	Q	Q	Y	B	—	—	S	T	Y	R	V	V	S	T	V	L	T	V	L	H	Q	N	W	L	—	15		
7 C _γ 3		H	T	B	I	S	Z	S	H	P	B	—	—	A	T	F	S	A	V	G	E	A	S	I	C	T	E	B	B	W	N	—	20		
8 C _γ 3		S	T	R	K	E	E	K	Q	R	S	D	—	—	G	T	L	T	V	T	S	T	L	P	V	D	K	S	R	W	Q	—	19		
9 C _γ 4		K	T	T	P	P	V	L	D	S	B	—	—	G	S	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	—	19			
10 C _γ 4		V	T	S	A	P	M	P	E	P	Q	A	P	—	G	R	Y	F	A	H	S	I	L	T	V	S	E	E	E	W	N	—	22		
11 C _γ 4		S	T	T	Q	P	R	K	T	K	G	—	—	S	G	F	F	V	F	S	R	L	L	E	V	S	R	A	E	E	W	Q	—	17	
HLA chains																																			
12 β _{2m}		E	H	S	D	L	S	F	S	K	D	—	—	W	S	F	Y	L	L	Y	S	Y	T	E	F	T	P	T	—	—	—	19			
13 B7, ac-2		E	L	V	E	T	R	P	A	G	D	—	—	R	T	F	E	K	W	A	A	V	V	V	P	S	G	—	—	—	—				
(a)																																			
(b)																																			
Human Ig C domains		100										110										120													
Light chains																																			
1 kappa		—	K	H	K	V	Y	A	C	E	V	T	H	Q	G	L	S	S	P	—	—	V	T	K	S	F	N	R	G	E	C	—	22		
2 lambda		—	S	H	R	S	Y	S	C	Q	V	T	H	E	G	—	—	S	T	—	—	V	E	K	T	V	A	P	T	E	C	S	20		
Heavy chains																																			
3 C _γ 1		G	T	Q	—	T	Y	I	C	N	V	N	H	K	P	G	S	N	T	K	—	—	V	D	K	R	V	E	P	K	S	C	/	17	
4 C _γ 1		G	T	N	S	H	V	V	C	K	V	Z	H	P	B	G	T	B	T	F	K	Q	—	—	E	K	D	V	—	P	L	P	—	14	
5 C _γ 2		—	S	Q	E	M	E	T	C	R	V	S	H	K	A	G	L	P	S	A	P	—	—	I	E	K	S	S	M	C	V	P	—	24	
6 C _γ 3		—	D	G	K	E	Y	T	C	K	V	T	S	N	K	A	L	P	S	P	—	—	I	K	Q	T	I	S	R	K	P	K	G	—	15
7 C _γ 3		—	S	G	E	R	F	T	C	K	V	T	H	P	H	L	P	R	A	—	—	L	M	R	S	T	T	K	T	S	P	G	—	20	
8 C _γ 3		—	E	G	E	T	Y	Z	C	R	V	T	H	P	H	L	P	R	A	—	—	L	M	R	S	T	T	K	T	S	P	G	—	19	
9 C _γ 4		—	Q	G	N	V	F	S	C	S	V	M	H	E	A	L	P	H	N	R	Y	T	—	Q	K	S	L	S	L	S	T	G	K	—	19
10 C _γ 4		—	T	G	E	T	Y	T	C	V	V	A	H	E	A	L	P	S	—	—	V	—	Q	R	A	V	S	V	N	P	G	K	—	22	
11 C _γ 4		—	E	K	D	E	F	I	C	R	A	V	H	E	A	A	S	P	S	Q	T	V	—	Q	R	A	V	S	V	N	P	G	K	17	
HLA chains																																			
12 β _{2m}		—	E	K	D	E	Y	A	C	R	V	N	H	V	T	L	S	Q	P	K	—	I	V	K	—	W	D	R	D	M	—	—	19		
13 B7, ac-2		—	E	E	Q	R	Y	T	C	H	V	Q	H	E	G	L	P	K	P	L	T	/													
(a)																																			
(b)																																			

appear in at least 80% of the Ig constant domains shown in Fig. 2), six also appear in ac-2 (Cys 29, Pro 36, Trp 45, Cys 98, Val 100 and His 102). In all, there are 30 positions in the Ig constant domain at which one residue appears in at least 50% of the sequences, the ac-2 fragment matches 14 of these residues. All of these residues are marked in Fig. 2.

Another interesting feature of the homology between ac-2 and Ig domains is revealed when the homologous residues are related to the observations of Beale and Feinstein³³ concerning the role of side chains in domain folding. These authors aligned residues occupying analogous positions in the crystallographic structures³⁴⁻³⁶. The resulting alignment for Ig domains and β_2m resembles very much that of Fig. 2, suggesting that sequence homology data for a new member may contain information on its tertiary structure. From their comparison several highly conserved features of Ig domain sequences and relationships to domain folding were identified. Of particular interest is that several of these features can be found in the primary structure of the ac-2 fragment of HLA-B7. Thus a dominant feature of Ig constant domains is that at some positions, substitutions are restricted within a group of hydrophobic amino acids (Phe, Ala, Tyr, Pro, Met, Val, Leu and Ile). The side chains of some of these residues are found to fill the internal space within the domain. Of 19 such residues in Ig domains, 15 are found in ac-2 (see Fig. 2). In Ig, some of these conserved residues form three hydrophobic clusters³³ which may be important in the nucleation of Ig chain folding. Most of the residues participating in the formation of such clusters appear in ac-2. These include all three residues in the first cluster (Val 8, Ala 31 and Val 100), three out of four in the second (Phe 34, Ile 41 and His 102) and two out of three in the third (Leu 27 and Val 81). Beale and Feinstein³³ further noted that many of these conserved positions correspond to the stretches of alternating hydrophobic residues characteristic of the β -sheet structure in Ig domains. Four such stretches seem to appear in ac-2, at positions 25-34, 41-45, 75-83 and 96-100. These features are illustrated in Fig. 3, depicting the human C_λ folding in relation to the ac-2-Ig homology. Of the five residues conserved in the C_λ domain (Fig. 3) and not found in ac-2, two, Trp 88 and Pro 313, are located in the loop portion of C_λ . Gaps have been inserted in the ac-2 sequence around these positions (Fig. 2). Therefore, these loops, if present in ac-2, may be shorter than the corresponding segments in Ig. In the light of the above, the ac-2 fragment of HLA-B7 has the potential of folding in a manner similar to that in Ig domains. Such a suggestion is in agreement with the high percentage of β -pleated sheet in the HLA-B7 antigen as seen by circular dichroism³⁷.

The matrix of differences⁴⁴ derived from the alignments in Fig. 2 is shown in Table 1. From these data it may be seen that ac-2 displays the same pattern of differences in its relation to all Ig sequences as does any other sequence in Fig. 2. Thus, ac-2 is not related to any particular Ig domain. The ac-2 fragment and β_2m are slightly more different from Ig domains than the latter are among themselves. Also, ac-2 and β_2m are marginally more similar to each other than they are to Ig domains. As it is not clear whether such differences are significant, it may only be

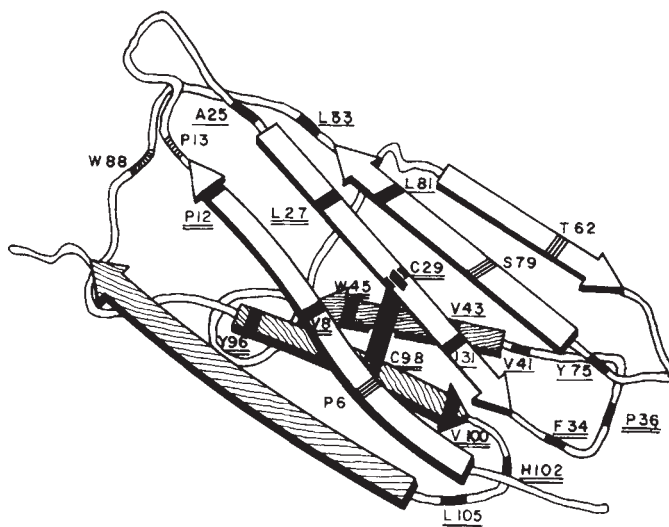


Fig. 3 The folding scheme of a constant Ig domain, as obtained by X-ray crystallographic studies of the Mcg λ human light chain dimer³⁶. Arrows denote the location and direction of strands in the β -pleated sheet structure (open, the four-stranded sheet; hatched, the three-stranded sheet). The dark bar between positions 29 and 98 represents the intradomain disulphide bond. Numbering is according to Fig. 2. Single letter coded⁴⁴ residues are those appearing in the Mcg light chain sequence, except L105 which appears in most Ig constant domains and ac-2 but is deleted in Mcg. Solid bars denote residues that are invariable in all or most Ig constant domains³² and appear in the second disulphide region of HLA-B7 (doubly underlined) or restricted to a group of hydrophobic residues in all of the above (underlined). Residues which are highly conserved in Ig constant domains³², but different in the second disulphide region of HLA-B7, are indicated by the hatched bars. Residues identified by the single letter code⁴⁴ are from the Mcg sequence.

concluded that the Ig-HLA and β_2m -ac-2 divergences occurred around the time of the C_H - C_L divergence and C_H domain duplication³².

To establish the statistical significance of the homology between ac-2 and Ig constant domains, the Z factors (as defined in ref. 28) for some pairs of sequences in Fig. 2 were calculated (Table 2). Such a procedure was used to classify proteins into superfamilies representing a minimal level of relationship between sequences³¹. Values $Z > 6$ suffice to indicate that any two sequences belong to the same superfamily, while $Z > 3$ is sufficient when some functional relationship is known to exist between two proteins³¹. The Z values obtained for the ac-2 fragment with β_2m and seven Ig constant domains are greater than 6 (Table 2). Thus, ac-2 is a new member of the previously established superfamily of Ig constant domains and β_2m ²⁸. The Z values of ac-2 with a variable Ig domain are as low as those found in comparisons of Ig constant with variable domains (Table 2 and ref. 28).

Fig. 2 The alignment of the ac-2 fragment of HLA-B7 with the human-derived sequences of the superfamily of Ig constant domains and related proteins. Ig sequences from other species were not included since interspecies differences are smaller than those between different classes or homology regions³². A computer program AL04 (equivalent to ALIGN, ref. 30) was used, equipped with a core memory saving modification that enabled us to run it on a Data-General Nova 1200 minicomputer. AL04 is based on the algorithm of Needleman and Wunsch⁴⁵ which affords the systematic introduction of gaps in both sequences so as to maximise the homology score. As a criterion for the 'goodness of fit' of each matched pair of residues, a scoring matrix²⁷ was used, whose elements are related to the tendency of those residues to replace each other in many homologous proteins, as well as to their respective abundances and mutabilities. The actual matrix was an updated version for an evolutionary distance of 256 accepted point mutations per 100 residues⁴⁶. In the alignments presented, gap formation was controlled by a penalty of 2 for each break and a bias factor of 2 added to all matrix elements (compare ref. 30). As in all cases gaps appeared only in ac-2, it was possible to reproduce here both the sequences and the numbering system of alignment 27 of ref. 32. The ac-2 sequence was added based on representative alignments following the majority where differences occurred. Boldface letters signify residues at which a particular sequence is identical to ac-2 (ac-2 residues are all boldface irrespective of identities). The number of identities scored by each sequence is given in the extreme right column. Names of sequences designate the Ig heavy chain class and the homology region (for the γ chain, those are 1, 3 and 4). Conserved residues in the Ig sequences, including β_2m , are indicated in a by (-), occur in 6-7/12; (=), occur in 8-9/12; ($\equiv$), occur in 10-11/12 and ($\equiv$), occur in 12/12 sequences; b, ($\blacktriangle$), indicates positions of hydrophobic residues (Phe, Ala, Tyr, Pro, Met, Val, Leu, Ile or Cys) whose side chains are found to fill the internal space in a folded Ig domain³³; (*) indicates that an identical (a) or homologous (b) residue occurs in ac-2. Residues are indicated using the single letter code⁴⁴.

Table 2 Z factors

	ac-2	β_2m	C $_{\kappa}$	C $_{\gamma}4$
β_2m	8.4			
C $_{\kappa}$	8.2	4.1		
C $_{\gamma}4$	10.0	6.7	13.6	
C $_{\lambda}$	11.1	—	16.0	—
C $_{\gamma}1$	6.9	—	—	—
C $_{\mu}3$	7.2	—	—	—
C $_{\mu}4$	9.2	—	—	—
C $_{\epsilon}4$	6.4	—	—	—
V $_{\kappa}$	0.6	1.1	1.9	1.2

The statistical alignment scores for ac-2 and several Ig domains. The value of the statistical score, Z , is calculated according to ref. 28. In short, alignment scores were calculated as described in Table 1 legend for the pairs of sequences in question. Then, using the same alignment procedure and gap-controlling parameters, alignment scores were calculated also for one of the sequences with 50 randomisations of the other. $Z = (S - \mu) / \sigma$ where S is the score for the actual protein and μ and σ are the mean and standard deviation for the distribution of scores for randomised sequences. Z is a measure of how likely is a value S to arise by chance; for example, Z equal to 3 and 6 correspond to probabilities of 10^{-3} and 10^{-9} respectively, for this to occur. For comparison some Z values are given for pairs of Ig domains. (—), not determined. V $_{\kappa}$ is human AG variable Ig sequence reproduced from ref. 32.

Discussion

The ac-2 fragment of the heavy chain of HLA-B7 is approximately the same size as an immunoglobulin constant domain and, like the latter, contains a disulphide loop of 55 residues. The data presented here establish unequivocally that the heavy chain of HLA-B7 contains an Ig-like region. The features which relate the ac-2 sequence to that of Ig constant domains are summarised as follows: (1) The number of amino acid residues between the two cysteines is very similar to that found in Ig constant domains. (2) They are identical at the three invariant residues—the two cysteines and a tryptophan. (3) They have a high degree of homology at conserved hydrophobic residues. In Ig, some of these participate in the formation of clusters of hydrophobic side chains which are thought to be important in chain folding. (4) More homologous residues are found near the two cysteines of ac-2, as is the case when two different Ig constant domains are compared. And (5), short stretches of alternating hydrophobic residues occur and correspond to segments of β -pleated sheets in Ig. The latter points suggest that there may also be three-dimensional similarities between ac-2 and Ig constant domains. (6) Statistical analysis of the homolo-

gies between the ac-2 fragment of the HLA-B7 and Ig constant domains clearly show them to be members of the same superfamily, as defined previously²⁸. Such an assessment is the first quantitative indication that the HLA-B7 heavy chain is related to Ig.

One interesting conclusion which can be drawn from the sequence data is that there is an evolutionary relationship between the second disulphide loop region of HLA-B7 heavy chain and Ig constant domains. The occurrence of an Ig-like region in an MHC product suggests that the humoral and cellular arms of the immune system share a common ancestral development. Since cellular immunity phylogenetically predates humoral immunity³⁸, it would be extremely interesting to determine if primitive cellular responses involve HLA-A and -B-like proteins and eventually explore the structural nature of such proteins. Such studies should lead to a better understanding of the ancestral relationships between the two arms of the immune response. The sequence homology data presented here suggest that the HLA-Ig divergence as well as that of ac-2 and β_2m occurred around the time of Ig C $_{\text{L}}$ and C $_{\text{H}}$ divergence.

The presence of an Ig-like region in the heavy chain of HLA-B7 may have implications concerning the organisation of the genetic material encoding this polypeptide. In the mouse, a two gene—one polypeptide arrangement has been suggested for the H-2K and H-2D antigens. Both limited sequence data³⁹ and the sharing of a public specificity by each of the private specificities of a given haplotype⁴⁰ have been taken to indicate a two gene—one polypeptide arrangement for histocompatibility antigens. Considering the current state of knowledge of the organisation of Ig genes^{41,42}, the Ig-like region also adds credence to the possibility that a similar two gene—one polypeptide arrangement may exist for HLA-A and HLA-B heavy chains. The final elucidation of such questions must await additional amino acid sequence data of several specificities from multiple species and studies at the DNA level.

Using a tentative sequence of ac-2⁴³, A. Munro and A. Feinstein, independently (personal communication) noted that this region of HLA-B7 has structural similarities to an Ig constant domain.

Amino acid sequencing was performed utilising the facilities of the Protein Chemistry Laboratory at Harvard University. We thank M. L. Coe and T. Kostyk for technical assistance. This research was supported by NIH grant AI 10736. D.L. is a recipient of a Chaim Weizmann postdoctoral fellowship, J.A.L.C., a recipient of a PHS international research fellowship (1 FO5 TW 02582), and H.O. a recipient of a PHS research fellowship (5 F32 AI 05604).

Received 18 May; accepted 24 August 1979.

- Bach, F. H. A. *Rev. Genet.* **10**, 319–339 (1976).
- Paul, W. E. & Benacerraf, B. *Science* **195**, 1293–1300 (1977).
- McDevitt, H. O. (ed.). *Ir Genes and Ia Antigens* (Academic, New York, 1978).
- Zinkernagel, R. M. & Doherty, P. C. *J. exp. Med.* **141**, 1427–1436 (1975).
- McMichael, A. J., Ting, A., Zweerink, H. J. & Askonas, B. A. *Nature* **270**, 524–526 (1977).
- Shearer, G. M., Rehn, T. G. & Schmitt-Verulst, A. *Transplant. Rev.* **29**, 222–248 (1976).
- Dickmeiss, E., Soeborg, B. & Svejgaard, A. *Nature* **270**, 526–528 (1977).
- Grey, H. M. *et al. J. exp. Med.* **131**, 1608–1612 (1973).
- Nakamuro, K., Tanigaki, M. & Pressman, D. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2863–2865 (1973).
- Peterson, P. A., Rask, L. & Lindblom, J. B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 35–39 (1974).
- Silver, J. & Hood, L. *Nature* **249**, 764–765 (1974).
- Smithies, O. & Poulik, M. D. *Science* **175**, 187–189 (1972).
- Peterson, P. A., Cunningham, B. A., Berggård, I. & Edelman, G. M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1697–1701 (1972).
- Burnett, F. M. *Nature* **226**, 123–126 (1970).
- Gally, J. A. & Edelman, G. M. *A. Rev. Genet.* **6**, 1–46 (1972).
- Bodmer, W. F. *Nature* **237**, 139–145 (1972).
- Strominger, J. L. *et al. Transplant. Rev.* **21**, 126–143 (1974).
- Peterson, P. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 1612–1616 (1975).
- Terhorst, C., Robb, R. Jones, C. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4002–4006 (1977).
- Ferguson, W. S., Terhorst, C. T., Robb, R. J. & Strominger, J. L. *Molec. Immun.* **16**, 23–28 (1979).
- Parham, P., Alpert, B. N., Orr, H. T. & Strominger, J. L. *J. biol. Chem.* **252**, 7555–7567 (1977).
- Capra, J. D., Vitetta, E. S., Klapper, D. G., Uhr, J. W. & Klein, J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3661–3665 (1976).
- Tragardh, L., Wiman, K., Rask, L. & Peterson, P. A. *Scand. J. Immun.* **8**, 563–568 (1978).
- Strominger, J. L. *et al. Fedn Proc.* **37**, 1467 (1978).
- Lopez de Castro, J. A. *et al. Biochemistry* (in the press).
- Orr, H. T., Lopez de Castro, J. A., Lancet, D. & Strominger, J. L. *Biochemistry* (in the press).
- Dayhoff, M. O., Eck, R. V. & Park, C. M. in *Atlas of Protein Sequence and Structure* Vol. 5 (ed. Dayhoff, M. O.) 89–99 (National Biomedical Research Foundation, Washington, DC, 1972).
- Barker, W. C. & Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* Vol. 5 (ed. Dayhoff, M. O.) 101–110 (1972).
- Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 1 (ed. Dayhoff, M. O.) 1–8 (1973).
- Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 2 (ed. Dayhoff, M. O.) 1–8 (1976).
- Dayhoff, M. O., Barker, W. C. & Hunt, L. T. in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 2 (ed. Dayhoff, M. O.) 9–19 (1976).
- Barker, W. C. & Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* Vol. 5, Suppl. 2 165–190 (1976).
- Beale, D. & Feinstein, A. Q. *Rev. Biophys.* **9**, 135–180 (1976).
- Pojak, R. J., Amzel, L. M., Chen, B. L., Phizackerley, R. P. & Saul, F. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3440–3444 (1974).
- Davies, R. D., Padlan, E. A. & Segal, D. M. in *Contemp. Topics Molec. Immun.* **4**, 127–155 (1975).
- Edmundson, A. B., Ely, K. R., Ahola, E. E., Schiffer, M. & Panagiotopoulos, N. *Biochemistry* **14**, 3953–3961 (1975).
- Lancet, D., Parham, P. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3844–3848 (1979).
- Marchalonis, J. J. (ed.). *Comparative Immunology* (Wiley, New York, 1976).
- Vitetta, E. S. & Capra, J. D. *Adv. Immun.* **26**, 147–193 (1978).
- Démant, P., Iványi, D., Neauport-Sautes, C. & Snoek, M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4441–4445 (1978).
- Seidman, J. G., Leder, A., Nau, M., Norman, B. & Leder, P. *Science* **202**, 11–17 (1978).
- Sakano, H. *et al. Nature* **277**, 627–633 (1979).
- Ploegh, H., Orr, H. T., Robb, R. & Strominger, J. L. in *Biological Markers of Neoplasia* (ed. Ruddle, H.) 201–211 (Elsevier, New York, 1978).
- Dayhoff, M. O. (ed.). *Atlas of Protein Sequences and Structure* Vol. 5, D1–D6 (1972).
- Needleman, S. B. & Wunsch, C. D. *J. molec. Biol.* **43**, 443–453 (1970).
- Schwartz, R. M. & Dayhoff, M. O. in *Origin of Life* (ed. Noda, H.) 457–469 (Center for Academic Pub. Japan/Japan Sci. Soc. Press, Tokyo, 1978).

letters

A compact group of four QSOs with two appearing physically associated

C. Hazard*, H. C. Arp† & D. C. Morton‡

* Institute of Astronomy, Madingley Road, Cambridge, UK

† Hale Observatories, Carnegie Institution of Washington, California Institute of Technology, Pasadena, California 91103

‡ Anglo-Australian Observatory, PO Box 296, Epping N.S.W. 2121, Australia

The objective prism plates from the UK Schmidt telescope taken under good seeing conditions can reach to about magnitude +20 and provide ideal material for studies of the distribution over the sky. We have carried out a detailed search of one of these plates centred on RA 11 h 40 min, dec. 11°00' N in a preliminary investigation of QSO clustering on scales up to several arc min. Most of the 200 or so QSO candidates noted during this search are emission line objects and definite QSOs but several show no obvious emission features and were selected solely on the basis of their noticeably blue continuum spectra. Subsequent high-resolution spectroscopic observations show that while some of these blue continuum objects are low redshift QSOs a significant fraction are white dwarfs and blue compact galaxies. Allowing for these misidentifications we estimate that a total of 200 QSOs (~six QSOs per square degree) is a conservative upper estimate for the number of genuine QSOs to be found on this plate using our particular selection criteria. With a surface density of about six QSOs per square degree, pairings of QSOs at separations of several arc min are relatively common on a single plate and an individual pair is significant at the 1% level only at a separation of ≤ 10 arc. To be considered significant in its own right, a single association at separations of the order of 1 arc min requires a grouping of at least three objects. We report here the most striking association we noted satisfying these conditions, a compact group of four QSOs within a diameter of about 4 arc min. A physical association between two of the QSOs is suggested.

The field of this group of QSOs (denoted a, b, c and d) is shown in Fig. 1. The fifth object (e) is the radio QSO 1146+111 which has a redshift $z = 0.86$ (ref. 1); it shows no obvious emission features and from the objective prism data alone would have been considered only as a possible QSO candidate. There are no other probable QSO candidates in the area shown. Positions accurate to 1 arc min are given for all five QSOs in Table 1 together with O-magnitudes measured from the Palomar Sky Survey prints². Adopting a value of 200 for the total number of QSOs in the 36 square degree plate and assuming them to be randomly distributed, the estimated probability of finding, on this one plate, four or more QSOs within a 12 square arc min area is $\leq 6 \times 10^{-5}$. This represents only about a 10% probability of finding such a grouping in a search of the whole sky and strongly suggests that the group is by no means a chance association. An objection to this estimate is that it is strongly influenced by the choice of a 12 square arc min area which has been chosen to fit the particular association. However, when the

group was first discovered, the main reason for our believing that the association could be physically significant was the remarkable similarity of the objective prism spectra of a, b and c, all three of which show a single emission feature at roughly the same position in the spectrum, thus suggesting that they might have similar redshifts. Object d, however, which shows two clear emission lines which were identified as Ly- α and CIV at a redshift $z \approx 2$, had clearly a different redshift to other members of the group.

Spectroscopic observations on the five objects in Table 1 were carried out in January 1979 with the 200-inch telescope on Mount Palomar. The results of these observations are summarised in Table 2. Some preliminary observations at the Anglo-Australian Telescope in February on b, c and d gave results which agree with the Palomar observations but observations scheduled in April to obtain more detailed spectra were unfortunately clouded-out. The spectroscopic observations confirm the general inferences drawn from the objective prism data (although object a has clearly varied in brightness since the epoch of the Sky Survey), and strongly support the idea that objects a and c at least do not represent a chance association. Although the signal-to-noise ratios of our data are too low for an accurate comparison of their spectrum, the separation of 2.5 arc min is far too large to allow them to be interpreted as gravitational lens images, the favoured interpretation of the pair of QSOs 0957+561 A, B, which also have identical redshifts but are separated by only 6 arc s (ref. 3). The best redshift estimates

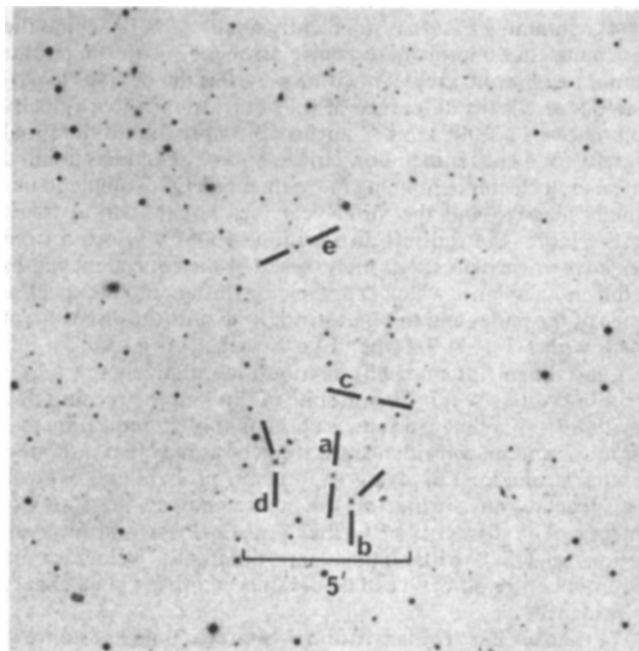


Fig. 1 An enlargement from the National Geographic Society-Palomar Sky Survey O-print of the region around the group of QSOs (a, b, c and d). Object e is the radio QSO 1146+111. The 5 arc min scale shown corresponds to the angle subtended by 1 Mpc at a redshift $z = 1$. ($H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$, $q_0 = 1$). Reproduced courtesy Hale Observatories.

Table 1 Positions and magnitudes (epoch 1950) from the Palomar Sky Survey Prints

Object	Right ascension			Declination			O-magnitude*
	h	min	s	h	min	s	
a	11	46	09.84	11	04	38.0	18.5
b	11	46	07.58	11	03	49.4	17.9
c	11	46	04.92	11	06	57.1	18.6
d	11	46	16.96	11	05	05.8	17.6
e	11	46	13.42	11	11	39.9	17.9

* Magnitudes are accurate to ± 0.3 mag.

Table 2 Spectroscopic data

Object	Magnitude* (V_{5000})	Redshift z	Emission lines observed
a	19.5	1.01 (1.010 ± 0.001)†	C III], [C II], Mg II
b	18.0	1.10	C III], [C II], [Ne IV], Mg II
c	18.9	1.01 (1.011 ± 0.001)†	C III], [C II], [Ne IV], Mg II
d	18.1	2.12	Ly- α , O I, [O IV], C IV, C III]
e	18.5	0.86	[C II], Mg II, He II

* Because of poor photometric conditions the magnitudes are accurate only to a few tenths of a magnitude.

† The figures in braces are the best redshift estimates for objects a and c.

for a and c give a maximum projected velocity difference of 450 km s^{-1} , while the angular separation of ~ 2.5 arc min corresponds to a projected physical separation of 500 kpc ($H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$, $q_0 = 1$). These are typical values for cluster galaxies and the natural inference is that a and c are both members of the same galaxy cluster. Their discovery therefore indicates that the QSO phenomenon is not confined to one highly peculiar galaxy in a cluster.

The status of object b is not clear, for the probability of finding an additional two QSOs within a 5 arc min circle ($\sim 1 \text{ Mpc}$ at $z = 1$) containing a and c is sufficiently small ($\leq 5 \times 10^{-4}$) that the possibility that one of these is also associated with the cluster cannot be ignored. Object b with $z = 1.10$ is the obvious candidate but a redshift difference of 0.09 corresponds to a velocity difference of $13,000 \text{ km s}^{-1}$, an unacceptably high differential velocity for a cluster member. However, as T. Gold has pointed out to us, a cluster containing more than one QSO might be unusually massive and the virial velocities larger than average. Furthermore, the redshift distribution of QSOs selected from objective prism plates is strongly biased by the equivalent widths of the emission lines which happen to be shifted into the spectral range of the plates and this bias strongly favours the discovery of QSOs with $1.7 \leq z \leq 3.2$ when Ly α is visible. As a result of this bias and the intrinsic redshift distribution, relatively few QSOs are found with $z \leq 1.7$. That in spite of this bias the redshift of b is within 10% of that of a and c is perhaps another indication that it is not a random coincidence. As it can be argued that this is just another example of applying 'a posteriori' probabilities we will not speculate any further on this matter nor on the status of object e. The discovery of further statistically significant associations of QSOs with similar large differential velocities are required before object b can be considered other than a chance coincidence.

We thank Misses F. Hazard and F. Short for their assistance in searching the objective prism plate and for measuring the accurate optical positions.

Received 27 April; accepted 25 September 1979.

1. Smith, H. E. *et al. Astrophys. J.* **215**, 427 (1977).
2. Hayman, P. G., Hazard, C. and Sanitt, N. *Mon. Not. R. astr. Soc.* (in the press).
3. Walsh, D., Carswell, R. F. & Weymann, R. J. *Nature* **279**, 381 (1979).

IUE UV spectra of the clumpy irregular galaxy MKN297

P. Benvenuti*, C. Casini† & J. Heidmann‡

* ESA Villafranca Satellite Tracking Station, Madrid, Spain

† Istituto di Astronomia and Istituto di Fisica dell' Università, Milan, Italy

‡ Observatoire de Paris, 92190 Meudon, France

Clumpy irregular galaxies are made up of 5–10 bright clumps loosely scattered in a common envelope, have excess near-UV radiation and are giant compared with classic irregular galaxies^{1,2}. Clumps are apparently ~ 100 times more massive than giant H II regions such as 30 Doradus or NGC604 (ref. 3). To get an insight into their properties we have obtained UV spectra using the IUE satellite of one of the clumpy irregular galaxies, MKN297. The UV continuum spectrum thus obtained follows a power law and is steeper (fainter at short wavelengths) than that of a classic irregular or late galaxy. The 155 nm intrinsic luminosity is 400 times larger than that of 30 Doradus. The observed absorption lines can be attributed to early O or B stars, and no emission lines are detectable. We report here that the spectra indicate that each of the clumps contain $\sim 10^4$ early stars and are supergiant H II regions in which exceptional bursts of star formation take place.

We have obtained two low dispersion spectra (0.6 nm resolution), one at short ($\lambda\lambda$ 115–195 nm) and one at long ($\lambda\lambda$ 190–320 nm) wavelengths, using exposure times of 290 and 310 min. The 10×20 arc s slit was used at PA 27° . The central clump, about half of each of the two next strongest clumps and a few other fainter clumps fell within the field of view of the slit. Without a photometric map of MKN297 it is difficult to evaluate the proportion of light which enters the slit; from Arp's photograph⁴ we assume that 50% light and five clumps are present.

The main features of MKN297 (NGC6052) which have already been investigated^{3,5–12} are given in Table 1.

Figure 1 shows the UV spectrum smoothed with a 5 nm band and Fig. 2 shows two unsmoothed sections. The continuum has the shape of a power law; when corrected for absorption in our Galaxy with Nandy *et al.*¹³ reddening curve, the spectral index is -1.69 . It is steeper than those observed for late galaxies or classical irregular galaxies, such as the Large Magellanic Cloud¹⁴ and NGC4449 (ref. 15).

The colours, neutral hydrogen content, H β flux and radio continuum flux densities allows a few magnitudes to be estimated for the internal absorption in MKN297; however, these values are not enough to flatten the spectrum.

A steep UV spectrum can be accounted for by an *ad hoc* star population. The absorption lines in our spectrum indicate that

Table 1 Physical properties of MKN297

				Refs
Observed velocity	V_x	4,700	km s^{-1}	12
Galactic extinction in B system	A_B	0.31	mag	18
Visual magnitude	V	13.18	mag	12
Colours	$B - V$	0.44		12
	$U - B$	-0.37		12
	$V - R$	0.56		12
H I flux	F_H	2.5	$10^6 \odot \text{ Mpc}^{-2}$	3
Apparent diameter	a_0	2.0	arc min	3
Distance	D	64	Mpc	
Absolute H β luminosity		24.0	$10^{40} \text{ erg s}^{-1}$	11
Absolute [O III] luminosity		71.6	$10^{40} \text{ erg s}^{-1}$	11
Flux density at 1.4 GHz		166	mJy	10
Flux density at 10.5 GHz		18	mJy	9

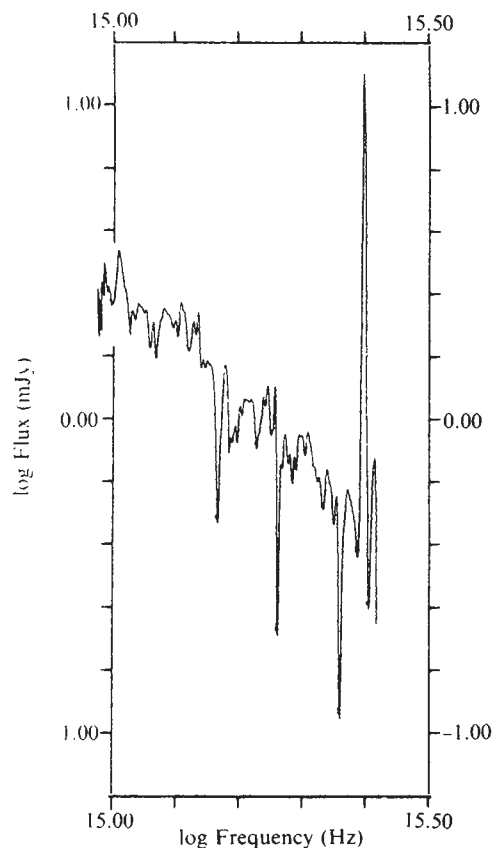


Fig. 1 Smoothed UV spectrum of MKN297. The high frequency spike is due to geocoronal $\text{Ly}\alpha$ and the three strong absorptions are resseau marks.

they are produced by stars earlier than AO and, though more like class V stars, they do not exclude class I. Thus, using UV spectra from the Jamar *et al.*¹⁶ Catalogue, corrected for galactic absorption¹³, we see in Fig. 3 that fits to the UV spectrum of MKN297 can be obtained by: (i) 3.0×10^4 O7 V stars at the high frequency end; (2) 1.3×10^9 A5 V stars combined to the above O7 V stars for wavelengths up to 200 nm; (3) 1.5×10^5 B5 to B8 I stars for most of the UV range.

An attempt has been made to fit the spectrum with a population of O7 and AO V stars plus an internal reddening. The main conclusion is that the stellar flux cannot be reddened using the 'normal' curve of Nandy *et al.*¹³ because, even at low colour excesses, it produces a detectable 2,200 Å absorption that is not present in our spectrum. This can be explained if the 2,200 Å

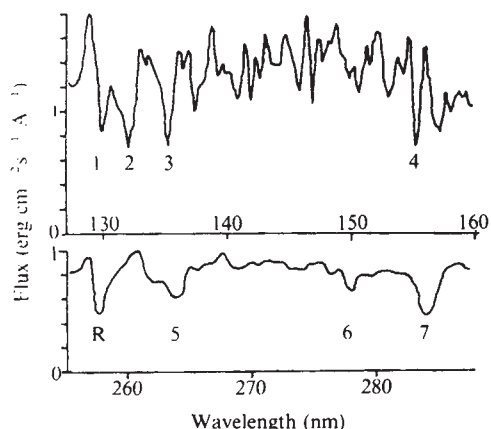


Fig. 2 Two sections of the 'net' spectrum in the short and the long wavelengths. The numbers 1–7 are those given in Table 2 for identification purpose. R is a resseau mark.

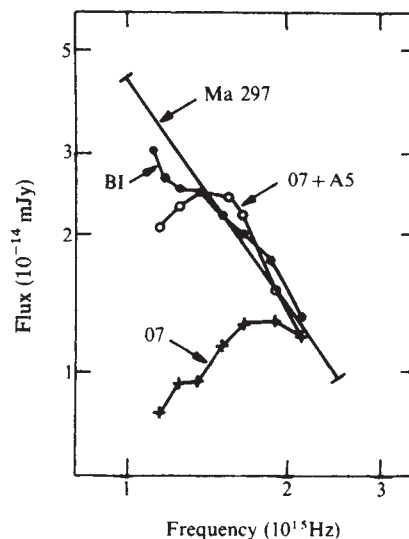


Fig. 3 The UV spectrum of MKN297, corrected for absorption in our Galaxy (straight line) compared with the different star populations given in the text.

feature were mainly due to dust scattering and if we took into account that we are observing the whole region where the scattering takes place. Allowing for this, a reasonable fit has been obtained with a mixture of 4×10^5 O7 stars, 2.5×10^9 AO V stars and an internal reddening of $E(B - V) \sim 0.5$.

Therefore, a clump apparently contains $\sim 10^4$ early stars.

An interesting result is obtained by comparing the UV emission of MKN297 with that of the giant H II region 30 Doradus. Using the 155 nm map of 30 Dor obtained by Korneef¹⁷, the de Vaucouleurs *et al.*¹⁸ galactic absorption and the Nandy *et al.*¹³ curve one gets a flux density of 30 Dor corrected for galactic absorption which, when transferred from its 50 kpc distance to that of MKN297, is 400 times less than the one we observed for MKN297. A clump is thus ~ 100 times more powerful than a giant H II region.

Absorption lines together with an indication of their quality are listed in Table 2. Two lines may be attributed to interstellar absorption in our galaxy: O I $\lambda\lambda 130.1$ and 135.2 and C IV $\lambda 155.0$ nm. The others correspond to the redshift of MKN297.

Spectral features typical of stars from late O to AO are visible: Si IV $\lambda 140.0$, C II $\lambda 133.5$, C IV $\lambda 155.0$, Fe II (8) $\lambda 162.0$, Fe II (3) $\lambda 234.0$ nm.

Table 2 Identification of absorption lines in the UV spectrum of MKN297

Measured λ (Å)	Corrected λ (Å)	Identification (main contributors)	λ_0 (Å)	No. in Fig. 2	Comments
1,238	1,219	Ly α	1,216		
1,256	1,236	N V	1,241		
1,301	—	O I	1,302	1	Galactic
1,321	1,300	Si III	1,299	2	
1,352	1,331	C II	1,335	3	
1,408	1,386	Si IV	1,398		Doubtful
1,552	—	C IV	1,549	4	Galactic
1,570	1,546	C IV; Si II	1,549; 1,533		
1,646	1,620	Fe III; Al III; Fe II; He II	1,600; 1,640		Broad feature
1,701	1,675	Al II; Fe II	1,671; 1,675		
1,922	1,892	Fe III	1,886; 1,897		Several multiplets
1,948	1,918	Fe III			Doubtful
2,026	1,994	Fe III			Doubtful
2,340	2,304	?			Doubtful
2,373	2,336	Fe II; Cr II	2,340		Several multiplets
2,622	2,582	Fe II	2,578		Broad feature
2,640	2,599	Fe II	2,599	5	Broad feature
2,783	2,740	Fe II	2,739	6	
2,841	2,797	Mg II	2,799	7	
2,887	2,843	Mg I	2,852		

The Fe II and other singly ionised metal feature at $\lambda 240.0$ which is a strong feature in stars later than AO V is not visible. The Mg II $\lambda 280.0$ and Mg I $\lambda 284.0$ nm lines are not particularly strong, suggesting also types not later than A.

In the visible, du Puy and de Veny⁵ observe Ca II $\lambda 393.3$ nm and some Balmer lines, which also correspond to early types.

The identifications are consistent with the presence of hot main sequence stars. The $\lambda 234.0$ line favours class I–II stars, but has also been observed in class IV stars¹⁹. Other features corresponding to bright stars, Fe III (51) $\lambda 192.0$ and the wide dip from $\lambda 180$ to 215 (ref. 20) are dubious.

The presence of $\lambda 155.0$ and the absence of $\lambda 240.0$ are also consistent features for class I stars.

Using the criteria of Jaschek *et al.*²¹, the r_1 , r_2 , r_3 values calculated from flux densities at $\lambda\lambda 141.0$, 155.0 , 158.0 , 162.0 nm after correction for absorption in our galaxy yield an integrated spectral type B5 V. A further internal absorption $E(B - V) = 0.6$ would yield a type B2.

The r_5 ratio of flux densities at $\lambda\lambda 240.0$ and 274.0 nm of Cucchiari *et al.*²² for A stars leads also to a faint luminosity class.

There are no emission lines in our UV spectrum of MKN297, contrary to the visible case^{5,6}. With the hypothesis that the gas in the clumps has physical conditions similar to those of H II regions, the expected intensity ratio between, for example, the C III] 190.7 – 190.9 nm lines and the [O II] 372.7 – 272.9 ones is found to be too small to give a detectable signal at ~ 191 nm.

Received 13 July; accepted 11 September 1979.

- Casini, C. & Heidmann, J. *Astr. Astrophys.* **47**, 371–373 (1976).
- Casini, C. & Heidmann, J. *Astr. Astrophys. Suppl.* **24**, 473–493 (1976).
- Casini, C., Heidmann, J. & Tarengi, M. *Astr. Astrophys.* **73**, 216–221 (1979).
- Arp, H. C. *Astrophys. J. Suppl.* **14**, No. 123 (1966).
- du Puy, D. L. & de Veny, J. B. *Publ. Astr. Soc. Pacific* **87**, 637–642 (1969).
- Duflot, R. *Astr. & Astrophys.* **48**, 437–442 (1976).
- Sramek, R. A. & Tovmassian, H. M. *Astrophys. J.* **196**, 339–345 (1975).
- Sramek, R. A. & Tovmassian, H. M. *Astrophys. J.* **207**, 725–735 (1976).
- Kojoian, G., Sramek, R. A., Dickinson, D. F., Tovmassian, H. M. & Purton, C. R. *Astrophys. J.* **203**, 323–328 (1976).
- Sulentic, J. W. *Astr. J.* **81**, 582–594 (1976).
- Weedman, D. W. *Astrophys. J.* **171**, 5–12 (1972).
- Huchra, J. *Astrophys. J. Suppl.* **35**, 171–195 (1977).
- Nandy, K., Thompson, G. I., Jamar, C. & Monfils, A. *Astr. Astrophys.* **44**, 195–203 (1975).
- Henry, R. C., Feldman, P. D. & Fastie, W. G. *Astr. Astrophys.* **53**, 317–319 (1976).
- Code, A. D., Welch, G. A. & Page, T. L. *The Scientific Results from the Orbiting Astronomical Observatory* (ed. Code, A. D.) 559–574 (1972).
- Jamar, C. *et al. Ultraviolet Bright-star Spectrophotometric Catalogue* (ESA SR-27, 1976).
- Korneef, J. *Astr. & Astrophys. Suppl.* **29**, 117–121 (1977).
- de Vaucouleurs, G., de Vaucouleurs, A. & Corwin, H. J. Jr *Second Reference Catalogue of Bright Galaxies* (University of Texas Press, 1976).
- Hill, I. & Adelman, S. J. *Astrophys. J. Suppl.* **37**, 265–286 (1976).
- Thompson, G. I., Humphries, C. M. & Nandy, K. *Astrophys. J.* **187**, L81–L84 (1974).
- Jaschek, M., Jaschek, C. & Cucchiari, A. *Atlas of Ultraviolet Stellar Spectra* (Département Astr. Astrophys. Liège, 1979).
- Cucchiari, A. & Macau-Hercot, D. *Astr. Astrophys. Suppl.* **33**, 15–26 (1978).

Accretion powers the brightest stars

G. T. Bath

Department of Astrophysics, University of Oxford, South Parks Road, Oxford, UK

The brightest known stars in external galaxies are the Hubble–Sandage variables. These are normally assumed to be hot, massive, single stars evolving either towards or off the main-sequence. An alternative model is developed here involving binary mass transfer and accretion on to main-sequence stars. In this case the Hubble–Sandage variables are a third, and presumably final, class of binary in which accretion generates the bulk of the radiation observed. The other two classes are binaries containing an accreting white dwarf (cataclysmic variables)^{1,2} and an accreting neutron star or black hole (binary X-ray sources)^{3,4}. Accretion disk models developed in these two cases can be scaled directly to treat the main-sequence case. One

of the most complex objects in our own Galaxy is the η Carina nebula, rivalling in this respect the Orion Nebula and the Crab. I suggest below that η Car itself is an accreting main sequence star whose explosive outburst in the nineteenth century was caused by runaway mass accretion.

The Hubble–Sandage variables form a class of luminous, blue, irregular variable stars. In the original Hubble–Sandage study⁵ five variables in M31 and M33 were investigated to determine their group properties for use as distance indicators. The spectroscopic properties of these variables have been extensively studied^{6,7}, and the optical variability of eight group members has been discussed elsewhere⁸.

The main class characteristics are high luminosity, blue colour indices and irregular variability on time scales ~ 1 yr with amplitude $\lesssim 2$ mag. The maximum visual magnitudes are between -8 and -11 (visual luminosities $\sim 10^{39}$ erg s⁻¹). Gravities are low⁶. The spectra exhibit strong UV continua, no Balmer discontinuity and emission lines of H and He I, with generally weaker Fe II and [Fe II]. In this respect they closely resemble cataclysmic variables, particularly dwarf novae at quiescence. Dwarf novae show broad H and He I together with a strong UV continuum extending in some cases down to $1,250$ Å (refs 9, 10). Weak Fe II in emission is also observed⁹. The Balmer jump is either not observed, or, more rarely, is present weakly in emission^{9,11}. The principal difference is the increased breadth of the lines in dwarf novae, which are occasionally double. In contrast to the spectral similarities the luminosities of cataclysmic variables could hardly differ more. Rather than being the brightest stellar objects they are fainter than the Sun with luminosities $\sim 10^{32}$ – 10^{33} erg s⁻¹.

The general spectral similarity and the extreme disparity in luminosity suggest that similar physical processes occur in each class, but on differing scales. In quiescent novae the spectrum is generated by infall of gas from a companion onto a white dwarf mediated by an accretion disk. The Hubble–Sandage variables would result from similar physical conditions being generated but scaled to larger radii so that a much higher luminosity is generated. The occasional nova-like outbursts in Hubble–Sandage variables supports this identification.

I assume the Hubble–Sandage variables contain an accreting main-sequence star in a mass-transferring binary with a separation $\geq 10^{12}$ cm. This separation is needed to accommodate a Roche lobe-filling companion such that an accretion disk may be formed. With a main-sequence star the major changes affecting accretion result from the much reduced gravitational potential at the stellar surface, and the possibility of central masses exceeding $\sim 2M_{\odot}$. Recent work^{12–14} shows that with high accretion rates the main-sequence star will expand, often dramatically. Such expansion would require higher accretion rates to generate disk conditions similar to those derived below for normal main-sequence stars.

For an accretion rate $\dot{m}$ g s⁻¹ the gravitational luminosity, $L = GM\dot{m}/R$. The characteristic source temperature is roughly the black-body temperature, $T = (L/4\pi\sigma R^2)^{1/4}$. For a main-sequence star to be at the same temperature as an accreting white dwarf and hence have the possibility of exhibiting similar general spectral features, the accretion rate must be increased by a factor $\dot{m}_{MS}/\dot{m}_{WD} = (R_{MS}/R_{WD})^3 (M_{WD}/M_{MS}) \sim 10^5$ – 10^7 (for masses $1 < M_{MS}/M_{\odot} < 100$) and the ratio of luminosities is then $L_{MS}/L_{WD} = (R_{MS}/R_{WD})^2 \sim 10^4$ – 10^6 . Thus an accreting main-sequence star with disk temperatures similar to those of quiescent cataclysmic variables will have a disk luminosity $\sim 10^{36}$ – 10^{39} erg s⁻¹.

I now examine this model using steady disk models (the consequences of time dependence are unimportant). Assuming the disk is optically thick, which is valid so long as $T > 10^4$ K, then⁴

$$T(R) = \left(\frac{3GM\dot{m}}{8\pi\sigma R_{MS}^3} \right)^{1/4} \left(\frac{R}{R_{MS}} \right)^{-3/4} \left(1 - \left(\frac{R}{R_{MS}} \right)^{-1/2} \right)^{1/4}$$

The maximum disk temperature occurs at a radius 40% larger than R_{MS} and is given by

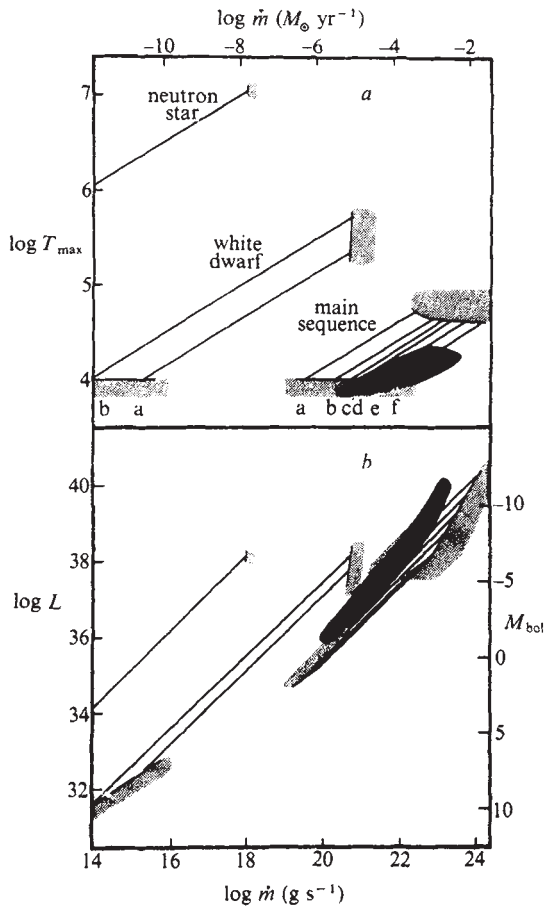


Fig. 1 Variation of maximum disk temperature (a) and disk luminosity (b) with accretion rate. Accretion tracks are shown for 0.5(a), 1(b), 3(c), 9(d), 30(e) and 121(f) solar mass main-sequence accretors. The region in which the accretion luminosity is less than the nuclear luminosity is indicated by heavy shading. At the upper boundary the disk luminosity is equal to the Eddington limit and the disk disrupts. At the lower boundary disk temperatures are less than $\sim 10^4$ K and the disk becomes optically thin. Tracks for white dwarfs of 0.4(a) and 1(b) $M_\odot$ and a neutron star of 1 $M_\odot$ are also shown.

$$T_{\max} = 0.488 \left(\frac{3GM\dot{m}}{8\pi\sigma R_{\text{MS}}^3} \right)^{1/4} \\ = 8.3 \times 10^3 \dot{m}_{20}^{1/4} \left(\frac{M}{M_\odot} \right)^{1/4} \left(\frac{R_{\text{MS}}}{R_\odot} \right)^{-3/4}$$

where $\dot{m}_{20} = \dot{m}/(10^{20} \text{ g s}^{-1})$. Disk temperatures are hotter than 10^4 K for accretion rates $> 10^{20} \text{ g s}^{-1}$.

Figure 1a shows the variation of $T_{\max}$ with $\dot{m}$ for main-sequence stars with masses in the range 0.5–121 $M_\odot$. (Radii are from refs 15–17.) For comparison disk temperatures are also shown for white dwarf and neutron star accretion.

Cut-offs exist at both low and high temperatures. With low accretion rates and temperatures $< 10^4$ K the rapid fall in opacity associated with hydrogen recombination leads to the possibility that the disk is optically thin. In these conditions radiation will be emitted in emission lines, with no strong continuum. In the case of massive early-type accretors a second cut-off exists at low temperatures. For $M > 9M_\odot$ the nuclear luminosity can be greater than the accretion luminosity for temperatures $> 10^4$ K.

At high temperatures disk solutions break down when the luminosity becomes supercritical⁴ and radiation pressure disrupts the disk. This occurs when $L > L_{\text{ed}} = 4\pi GMc/K$. This upper boundary is shown in Fig. 1a for an electron scattering opacity K , and a hydrogen abundance $X = 0.7$. The associated luminosity changes are shown in Fig. 1b.

It is evident that accreting main-sequence stars give a satisfactory model of Hubble–Sandage variables. With accretion rates 10^{20} – 10^{24} g s^{-1} disk temperatures are the same (1 – 4×10^4 K) as quiescent dwarf novae. Luminosities, on the other hand, are $\sim 10^{38} \text{ erg s}^{-1}$. To achieve the highest luminosities ($> 10^{39} \text{ erg s}^{-1}$) massive main-sequence stars ($> 9M_\odot$) are required, with accretion rates $\sim 10^{23} \text{ g s}^{-1}$. Massive main-sequence accretors will form the brightest Hubble–Sandage variables.

Are other physical properties, besides temperature, such as optical thickness and density, similar for both main-sequence and white dwarf accretion? The optical depth, τ , at temperatures $\sim 3 \times 10^4$ K is given by Sunyaev and Shakura's regime c^4 , where

$$\tau = 7.5 \times 10^2 \alpha^{-4/5} \dot{m}_{20}^{1/5} \left(1 - \left(\frac{3R_g}{R} \right)^{1/2} \right)^{1/5}$$

Here α is the disk viscosity parameter, and R_g the Schwarzschild radius. As $\alpha < 1$, and $R_g \ll R$, $\tau > 7.5 \times 10^2 \dot{m}_{20}^{1/5}$. With $\alpha = 1$ main-sequence accretors have $\tau \sim 10^3$ and quiescent cataclysmic variables have $\tau \sim 10^2$. Both are optically thick and will have a similar appearance.

The surface density, Σ , of the disk is given by⁴,

$$\Sigma = 9.8 \times 10^{-6} \alpha^{-4/5} \dot{m}_{20}^{7/10} \left(\frac{M}{M_\odot} \right)^{1/2} \\ \times \left(\frac{3R_g}{R} \right)^{3/4} \left(1 - \left(\frac{3R_g}{R} \right)^{1/2} \right)^{7/10} \text{ g cm}^{-2}$$

Thus

$$\Sigma > 2.9 \times 10^{-11} \dot{m}_{20}^{7/10} R^{-3/4} \left(\frac{M}{M_\odot} \right)^{1/4} \text{ g cm}^{-2}$$

For the case $\alpha = 1$ main-sequence stars typically have $\Sigma \sim 10^{3-4} \text{ g cm}^{-2}$ and white dwarfs $\Sigma \sim 10^2 \text{ g cm}^{-2}$. Thus density differences may affect the spectrum and could account for the stronger Fe II lines in some Hubble–Sandage variables.

The broadening and doubling of the lines in cataclysmic variables are due to rotational Doppler shifts of material moving in the disk with Keplerian velocities. The maximum velocity at the stellar surface is $V = (GM/R)^{1/2}$, corresponding to an orbital period $P = 2\pi(R^3/GM)^{1/2}$. For main-sequence stars in the mass range $0.5 M_\odot < M < 120 M_\odot$, periods are in the range $1 < P < 14$ h and velocities $400 < V < 1,200 \text{ km s}^{-1}$. For cataclysmic variables, $10 < P < 60$ s and $2,000 < V < 5,000 \text{ km s}^{-1}$. Hence main-sequence line-broadening is 3–10 times less than that occurring in cataclysmic variables and is compatible with the observed difference in emission linewidth. Note that the maximum broadening will occur in the most massive, and most luminous objects. Doubling of lines would support the disk model.

The Balmer jump is absent due to the spectrum being composed of contributions from different radii in the disk, as in cataclysmic variables. The spectrum should obey the $F_\nu \propto \nu^{1/3}$ law²³ observed in dwarf novae¹⁰. In the two colour diagram both classes lie in roughly the same region, $U-B \sim -1.0$, $B-V \sim 0.0$, close to the black-body line.

The required accretion rates of 10^{20} – 10^{24} g s^{-1} (10^{-6} – $10^{-2} M_\odot \text{ yr}^{-1}$) can be achieved (in principle) by Roche lobe-filling companions, particularly if these are giants. Variability is due to fluctuations in the mass transfer rate, possibly due to instabilities driven by ionisation zone destabilisation within the companion's envelope.

At accretion rates above the critical luminosity, auto-regulation of the accretion flow sets in and the disk is disrupted in the inner region⁴. This leads to a fraction of the accreting gas being ejected in the form of an outflowing wind. The low gravitational potential at the surface of a main-sequence star means that this wind is likely to be optically thick. It will have the same general properties as the optically thick winds observed in explosions of classical novae¹⁸—increases in the mass ejection rate will generate expansion of the photospheric radius, a decrease in both effective temperature and the bolometric correction but an

increase in the visual luminosity, with little change in the bolometric luminosity. Outflow velocities will be lower than in classical novae: roughly the same order as the escape velocity from the stellar surface.

Consequently there will be two types of spectroscopic and colour change produced by variations in the accretion rate. In the subcritical regime, increased accretion rates produce increasing disk temperatures along with increasing luminosity—both visual and bolometric. In the supercritical regime the disk is surrounded by an outflowing optically thick wind and the bolometric luminosity is held close to the Eddington value, L_{ed} . Increases in the wind mass-loss rate due to further increased accretion now produce decreasing temperatures along with increasing visual luminosity. The emission line spectrum of the disk will be replaced by an absorption line spectrum formed in the outflowing envelope—similar to the F or A type spectrum observed in classical novae at outburst maximum.

Is there any evidence for this supercritical state? The photographic magnitude of M33 Var A fell by 3.5 mag (ref. 5) in the visual in 1950–52, rose by 1.0 mag in 1952, before finally falling off sharply. Hubble and Sandage note that the changes in colour index indicate that the bolometric luminosity changed by only 1.4 mag. The visual luminosity fell by ~ 60 while the bolometric luminosity fell by ~ 4 . This agrees with the expected changes due to a variable optically thick wind at Eddington luminosities.

η Carina is a close galactic relative to the Hubble–Sandage variables. It has broad spectral lines, with relatively strong Fe II and [Fe II]⁶. Davidson¹⁹ deduced a temperature of 3×10^4 K for the underlying star. Between 1836 and 1856 η Carina underwent a major outburst, followed by a subsidiary outburst in 1889. The early F absorption spectrum of 1893 was slowly replaced by the characteristic emission spectrum. The absolute magnitude at outburst was ~ -14 (ref. 20), several magnitudes brighter than normal classical novae. At present, a cool surrounding dust cloud²¹ heated by the underlying stellar source produces the bulk of the emission. The bolometric luminosity is still ~ -14 , but most of the emission is re-radiated IR from the dust shell. This high and constant luminosity, the spectral changes, the broader than average emission lines, and the nova-like explosion indicate that η Carina could contain a massive, main-sequence, accreting component in a binary which underwent a burst of supercritical accretion. Polarisation observations²⁰ of the nebula suggest that the dust is concentrated in an annulus of size 10^4 AU, and this has been interpreted as evidence of binary structure. This is the first observational indication of possible binary structure in this type of object. A search for binary eclipses in Hubble–Sandage variables with periods in the range weeks to years would test the binary model.

The bright galactic source MWC349 is a similar object⁷. Thompson *et al.*²² interpret optical and IR observations as a proto-planetary disk seen face-on. In terms of the structure of the luminous source their model closely resembles that described here. The main difference is their interpretation as a preplanetary disk rather than an interacting binary.

If the brightest known optical stars are powered by infall of material on to a main-sequence star, the importance of accretion as an astrophysical energy source would be established beyond its present applications to X-ray binaries, novae and active galactic nuclei and quasars²³. Such binaries should exist, and they would be classified as Hubble–Sandage variables when observed in external galaxies.

I thank J. Barrow and A. Edwards for helpful comments.

Received 9 July; accepted 18 September 1979.

1. Bath, G. T., Evans, W. D., Papaloizou, J. & Pringle, J. E., *Mon. Not. R. astr. Soc.* **169**, 447 (1974).
2. Pringle, J. E. *Mon. Not. R. astr. Soc.* **178**, 195 (1977).
3. Pringle, J. E. & Rees, M. J. *Astr. Astrophys.* **21**, 1 (1972).
4. Shakura, N. J. & Sunyaev, R. A. *Astr. Astrophys.* **24**, 337 (1973).
5. Hubble, E. & Sandage, A. *Astrophys. J.* **118**, 353 (1953).
6. Humphreys, R. M. *Astrophys. J.* **200**, 426 (1975).
7. Humphreys, R. M. *Astrophys. J.* **219**, 445 (1978).
8. Rosino, L. & Bianchini, A. *Astr. Astrophys.* **22**, 453 (1973).
9. Warner, B. *IAU Symp.* No. 73, 85 (eds Eggelton, P., Mitton, S. & Whelan, J. A. J.) (Reidel, Dordrecht, 1976).

10. Bath, G. T., Pringle, J. E. & Whelan, J. A. J. *Mon. Not. R. astr. Soc.* (in the press).
11. Kraft, R. P. *Astrophys. J.* **135**, 408 (1962).
12. Ulrich, R. K. & Burger, H. L. *Astrophys. J.* **206**, 509 (1976).
13. Kippenhahn, R. & Meyer-Hofmeister, E. *Astr. Astrophys.* **54**, 539 (1977).
14. Neo, S., Miyaji, S., Nomoto, K. & Sugimoto, D. *Publ. astr. Soc. Jap.* **29**, 249 (1977).
15. Schwarzschild, M. & Härm, R. *Astrophys. J.* **128**, 348 (1958).
16. Iben, I. *Astrophys. J.* **142**, 993 (1965).
17. Stothers, R. *Astrophys. J.* **138**, 1074 (1963).
18. Bath, G. T. *Mon. Not. R. astr. Soc.* **182**, 35 (1978).
19. Davidson, K. D. *Mon. Not. R. astr. Soc.* **154**, 415 (1971).
20. Warren-Smith, R. F., Scarrott, S. M., Murdin, P. & Bingham, R. G. *Mon. Not. R. astr. Soc.* **187**, 761 (1979).
21. Pagel, B. E. J. *Astrophys. Lett.* **4**, 221 (1969).
22. Thompson, R. I., Strittmatter, P. A., Erickson, E. F., Witteborn, F. C. & Strecker, D. W. *Astrophys. J.* **218**, 170 (1977).
23. Lynden-Bell, D. *Nature* **233**, 690 (1969).

Streaming of interstellar grains in the Solar System

B. Å. S. Gustafson*† & N. Y. Misconi*

* Space Astronomy Laboratory, State University of New York at Albany, Albany, New York 12203

† Lund Observatory, University of Lund, Sweden

Deep-space probes offer excellent opportunities to study interstellar grains streaming into the Solar System. Direct detection of such particles would considerably add to our knowledge in many areas of cosmic dust research and could also provide information on the characteristics of both the interstellar medium and the interacting solar wind. Here we present the results of a theoretical study of the interaction between interstellar grains and the solar wind, including the effects of solar cycle variations in the interplanetary magnetic field. The latter is shown to influence significantly regions of concentration and depletion of interstellar grains within the Solar System.

Observations of the neutral gas in the solar vicinity suggest that the Solar System is moving relative to the local interstellar matter in a direction $\approx 7^\circ$ north of the solar equatorial plane (ecliptic longitude and latitude: $\lambda = 250^\circ$, $\beta = 7^\circ$)¹, and at a speed of ≈ 20 km s⁻¹ (ref. 2). Bertaux and Blamont³ computed number densities of interstellar grains in the solar cavity taking into account only gravitation and radiation pressure. Of the types of grains used in their model and based on their calculation of β (radiation pressure to gravitational attraction) only 0.02- μ m radius silicate grains were able to penetrate deeply inside the Solar System. They estimated the number density of these dust grains to be strongly peaked on a line extending from the Sun backwards in the downstream direction. This number density was found to be higher by a factor of ~ 100 than direct observations of dust grains by Alvarez⁴. In an attempt to clarify this difference, Levy and Jokipii⁵ made an order of magnitude estimate of the Lorentz force exerted by the solar wind magnetic field on a charged grain. They showed that if the 0.02- μ m radius silicate grains of Bertaux and Blamont carry a net charge of 5 V, then the Lorentz force strongly dominates, thereby destroying the gravitational focusing and suggesting that these grains are largely excluded from the Solar System.

Studies of interstellar grains suggest two populations of particles⁶: (1) elongated core-mantle particles of characteristic radius ≈ 0.12 μ m, with a ≈ 0.07 μ m radius core of silicate type material, and a rather complex mantle of molecular mixtures of mainly oxygen, carbon and nitrogen; and (2) ≈ 0.01 μ m thick silicate and/or graphite type grains. Bertaux and Blamont did not consider any of these populations. In the following calculations (see also, ref. 13) we show that although population (1) particles have $\beta > 1$, they still penetrate deep inside the Solar System.

The magnitude of the radiation pressure is given by $P_{\text{pr}} = (S/c)Q_{\text{pr}}$, where S is the flux, c is the speed of light, and Q_{pr} is the radiation pressure efficiency: $Q_{\text{pr}} = Q_{\text{abs}} + Q_{\text{sca}} (1 - \langle \cos \theta \rangle)$, where Q_{abs} and Q_{sca} are the efficiency factors for absorption and scattering, respectively, and $\langle \cos \theta \rangle$ is the asymmetry factor over

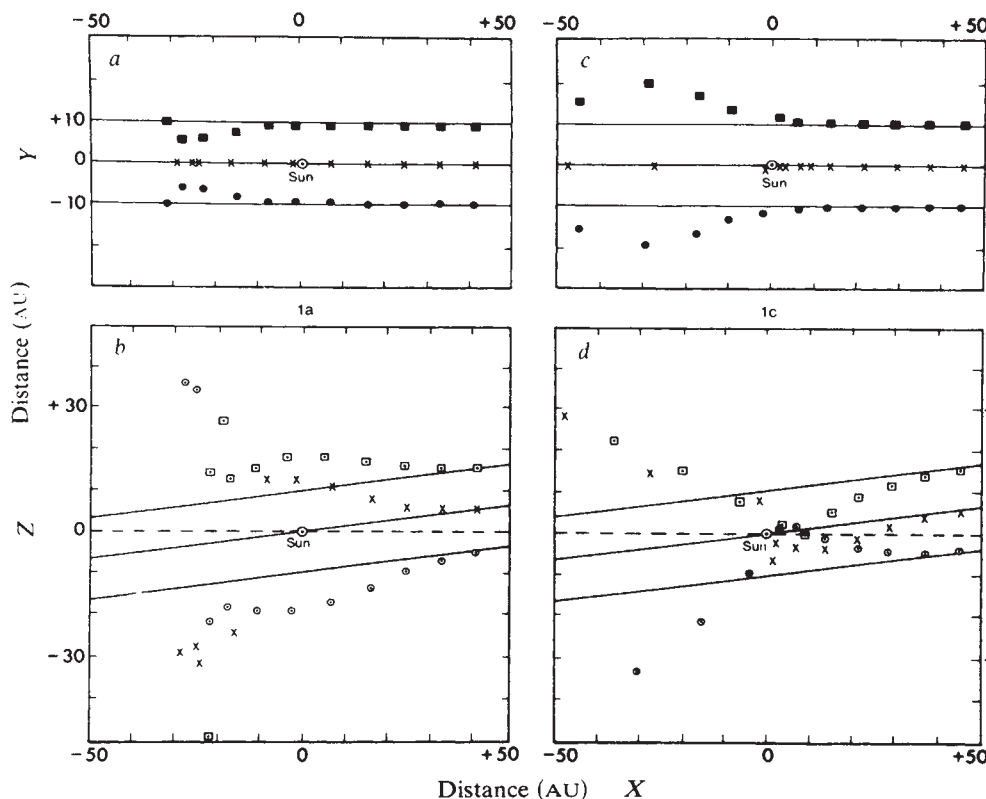


Fig. 1 Instantaneous positions of the $0.12\ \mu\text{m}$ core-mantle particles initially moving in the direction $\lambda = 70^\circ$, $\beta = 7^\circ$ along the solid lines. The particles are entering the Solar System at $X = +50\ \text{AU}$ with 2 yr interval. Three streams ($\blacksquare$, $\times$, $\bullet$) introduced at $Z = +6\ \text{AU}$ are projected onto the solar equatorial (X - Y) plane at the beginning (a) and in the middle (c) of a solar magnetic cycle. In b and d the three streams ($\square$, $\times$, $\circ$) introduced at $y = 0$ are similarly projected onto a plane (X - Z) perpendicular to the solar equatorial plane (dotted line).

the scattering angle θ . Scattering dominates over absorption for these dielectric particles in the scattering-efficient resonance region, and Q_{pr} was estimated using Mie theory for homogeneous spheres of equal volume and averaged core-mantle index of refraction. For a spherical silicate core coated by a concentric smooth spherical ice mantle representing particles of population (1) the computed Q_{pr} value using this approximation was found to be 0.4 ($\beta = 1.3$). As the grains that penetrate (population (1)) spend 30 yr or less in the Solar System, all radiation forces other than radiation pressure are negligible. Both solar-induced photoelectron emission and the accretion of solar wind ions will tend to charge these grains positively, whereas electron accretion produces negative charge. The equilibrium charge state is given by the relation

$$\sigma_e(V_0)f_e = \sigma_i(V_0)f_i + y\sigma_{\text{ph}}f_{\text{ph}} \quad (1)$$

where the σ s are the cross-sections of the grains for UV photons, ions and electrons, the f s are the corresponding fluxes, y is the photoelectron yield, and V_0 is the electrical potential of the grains. σ and y have been estimated for numerous silicates, and these lead to charges ranging from 5 to 12 V (refs 7, 8). The photoemission properties are not usually decreased by surface impurities or adsorbed layers⁷, or by amorphous structure⁸. However, for ice the work function is near the Lyman edge, and therefore photoemission is less efficient. We will adopt here +7 V for bare particles, and +1.2 V for the core-mantle grains. As the charged grains interact with the solar wind described by Svalgaard *et al.*⁹, they will be subject to a Lorentz force given by

$$F_L = \frac{q}{c} [(V_g \times B) - (V_{\text{sw}} \times B)] \quad (2)$$

where q is the grain's charge, V_g and V_{sw} are the speeds of the grain and of the solar wind, respectively, c is the speed of light and B is the magnetic field strength.

The magnetic field is torn in an archimedean spiral by the solar rotation. Thus

$$B_r = \frac{B_0}{r^2} \quad \text{and} \quad B_\Phi = \frac{-B_0\omega b^2(r-b)\sin\theta}{V_{\text{sw}}r^2} \quad (3)$$

where B_0 is the magnetic field strength at some reference distance ($b = 5$ – 10 solar radii), ω is the solar angular velocity, r is the heliocentric distance, Φ is the azimuth angle, and θ is the

heliocentric colatitude angle. The second (electrical) part of equation (2) which acts along θ will dominate as the grains enter the Solar System at low and medium heliocentric latitudes. This force, acting on a grain of given charge, is independent of the speed of a radially streaming solar wind as can be seen by substituting equation (3) in equation (2); high velocity plasma streams will not alter our results considerably.

The positive and negative magnetic fields are separated by a sinusoidal warped neutral sheet^{10,11}, the amplitude of which we assume to decay exponentially with increasing heliocentric distance. The speed of the solar wind as a function of θ was assumed to be of the form

$$V_{\text{sw}} = a + b |\cos\theta| \quad (4)$$

where a is the mean solar wind velocity at the equator ($400\ \text{km s}^{-1}$) and b is $300\ \text{km s}^{-1}$.

The grains also encounter other less important forces such as Coulomb drag due to the nearby passing solar wind electrons, which is given by

$$F_C = \frac{\pi s^2 N_e e^2 V_0^2}{m_e W^2} \ln \left(\frac{W^2 m_e P}{e V_0 s} \right) \quad (5)$$

where s and V_0 are the radius and charge of the grain, respectively, N_e , e , and m_e are the electron number density, charge and mass, respectively, and W is the velocity of the grains relative to the electrons. P is the impact parameter, which is assumed to be $\approx N_e^{1/3}$, following a poissonian distribution for the nearest neighbour distance. Another force is ion pressure resulting from impinging solar wind ions on the grains which is given by

$$F_i = \pi s^2 \bar{M}_i V F \quad (6)$$

where F is the flux of the solar wind ions, V is their velocity and $\bar{M}_i$ is the mean ion mass $\approx 2 \times 10^{-24}\ \text{g}$.

Trajectories of interstellar grains streaming into the Solar System were computed as follows: the grains are introduced at the boundary of the solar wind cavity ($X = 50\ \text{AU}$) at the beginning of the 22-yr solar magnetic cycle (defined as the cycle beginning when the solar dipole field acquires a south component). The equations of motion are integrated at small intervals of time (24 h), and the position, speed and acceleration of the particles are determined.

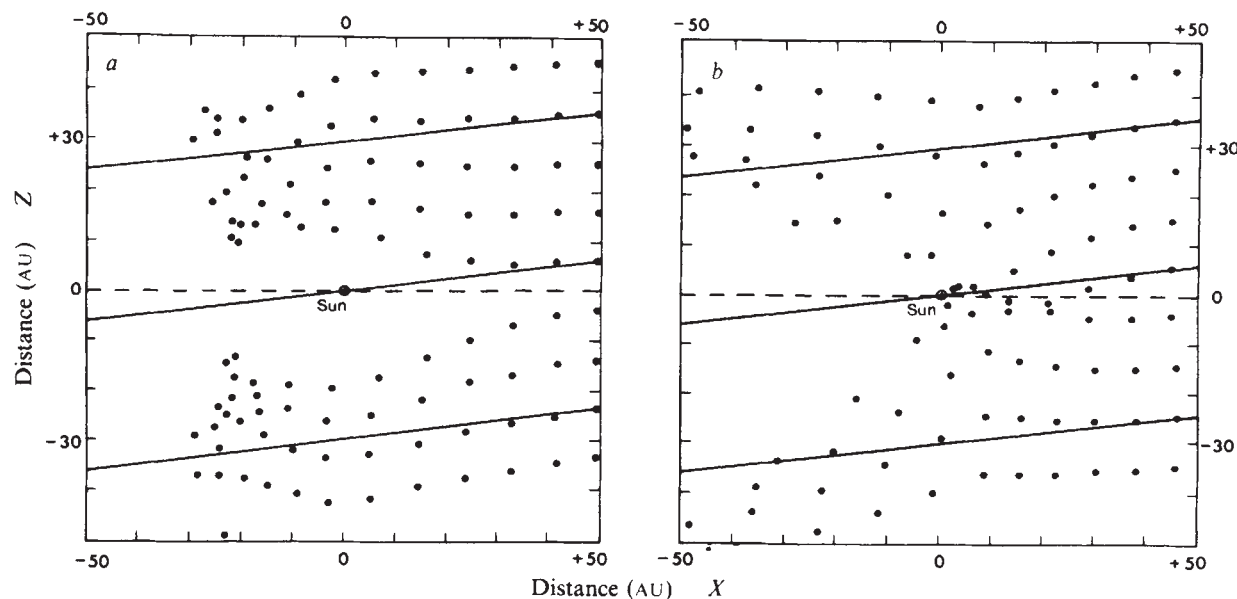


Fig. 2 Instantaneous positions of combined high latitude streams and the streams shown in Fig. 1b and d, at time zero (a) and time 11 yr (b) of a solar magnetic cycle.

The results of these computations are shown in Figs 1 and 2. Figure 1a and c shows the projection onto the solar equatorial plane (XY-plane) of the instantaneous positions of population particles arising from three streams which differ only by a separation of 10 AU in the initial value of their Y coordinate. Figure 1b and d shows the instantaneous positions in the XZ-plane of particles arising from three streams with $Y = 0$. One stream is initially directed towards the Sun and the other two lie parallel to and 10 AU on either side of that direction. The sequence of points belonging to a stream corresponds to positions of particles which differ by 2 yr from the initial time when each particle was introduced at the boundary of the solar wind cavity. Figure 1a and b shows the instantaneous positions of all the particles at a time corresponding to the beginning of the solar magnetic cycle. Figure 1c and d repeat this information for a time 11 yr into the solar magnetic cycle. The contrast between a, b and c, d clearly demonstrates the decisive influence of the solar magnetic cycle on the distribution of the grains. The streams are diverted towards the X-axis in Fig. 1a and away from that axis in Fig. 1c. Figure 1b shows that when the solar dipole field axis points north, the grains are diverted from the solar equatorial plane, mainly due to the action of the electrical part of the Lorentz force. The grains are concentrated towards this plane when the solar dipole field points south (Fig. 1d). Some 3 to 4 AU ahead of the Sun the streams cross the equatorial plane, creating an enhancement in the number density. The grains behind the Sun experienced the oppositely directed magnetic field of the first half of the solar magnetic cycle as they entered the solar wind cavity, thus creating a depletion zone in the wake of the Sun.

Our computations are almost insensitive to whether the reversal of the direction of the interplanetary magnetic field is instantaneous or whether it is as gradual as a sine function. These results are based on a simple dipole field where higher order multipole or asymmetrical magnetic fields are ignored.

In Fig. 2a and b additional streams, projected in the XZ plane, are introduced higher in latitude than the streams in Fig. 1b and d. Figure 2a shows a concentration of particles developing along a line $X \approx -20$ AU, $Z \approx \pm 10-20$ AU (peaked around $\phi \approx 180^\circ$). There is also a depletion zone of radius ≈ 20 AU surrounding the Sun. The deviation from the XZ-plane is $\leq \pm 0.1$ AU. The value of Q_{pr} used in the calculation is 0.4, which might be increased by surface roughness; however, this value decreases for elongated particles. The overall effect of increasing the radiation pressure efficiency Q_{pr} of the grains from 0.4 to as much as 1.0 ($\beta = 3.25$) is to move the concentration in Fig. 2b

≈ 3 AU along the positive X-axis. On the other hand, a decrease in the value of Q_{pr} has a negligible effect. The smaller bare grains that enter at low and medium heliocentric latitudes are essentially trapped around the solar wind magnetic field lines, and they are swept out near the solar wind cavity.

We have extended the study of the dynamics of interstellar grains to particles having radii of 0.12 and 0.005 μm streaming into the Solar System and included the effects of the solar dipole field's change of direction during the solar magnetic cycle. Additional forces on the particles such as Coulomb drag and solar wind ion pressure are less important than the Lorentz force. Particle trajectory computations show that an enhancement of the order of 50 or more in the number density of interstellar core-mantle particles may occur some 3–4 AU ahead of the Sun and close to the solar equatorial plane during yr 8–17 of the solar magnetic cycle. During the rest of the cycle, concentrations develop some 20 AU away from the Sun, while a depletion zone of ≈ 20 AU in radius surrounds the Sun.

As particle configuration varies strongly with the solar magnetic cycle, its effect must be taken into account in any treatment of this problem. Note that even grains with $\beta > 1$ will penetrate deep inside the Solar System during part of the solar magnetic cycle.

Subsequent computations may predict more precisely the directions and times to search for an interstellar component of the zodiacal cloud. Note that during the Solar Polar Mission (mid and late 1980s), concentrations of interstellar grains are expected (Figs 1d, 2b) which will offer a unique opportunity to detect the presence of interstellar grains in the Solar System¹².

We thank Drs J. M. Greenberg, J. R. Jokipii, E. H. Levy, K. F. Ratcliff and J. L. Weinberg for helpful comments, and Dr R. T. Wang for helpful discussions relating to radiation pressure efficiencies. This research was partially supported by the Planetary Atmospheres program of the NASA under grant NSG 7093.

Received 30 April; accepted 4 September 1979.

1. Weller, C. S. & Meier, R. R. *Astrophys. J.* **193**, 471 (1974).
2. Bertaux, J. L. *et al. Astr. Astrophys.* **46**, 19 (1976).
3. Bertaux, J. L. & Blamont, J. E. *Nature* **262**, 263 (1976).
4. Alvarez, J. M. *IAU Colloq.* No. 31 (1975).
5. Levy, E. H. & Jokipii, J. R. *Nature* **264**, 423 (1976).
6. Greenberg, J. M. & Hong, S. S. *IAU Symp.* No. 60 (eds Kerr, F. J. Simonson, S. C., III) (Reidel, Dordrecht, 1973).
7. Soumer, A. H. *Photoemissive Materials* (Wiley, New York, 1968).
8. Pierce, D. T. & Spicer, W. E. *Phys. Rev. Lett.* **27**, 1217 (1971).
9. Svalgaard, L., Wilcox, J. M. & Duvall, T. L. *Solar Phys.* **37**, 157 (1974).
10. Levy, E. H. *Nature* **261**, 394 (1976).
11. Schulz, M. *Astr. Space Sci.* **24**, 371 (1973).
12. Greenberg, J. M. & Schuerman, D. W. *Nature* **275**, 39 (1978).
13. Gustafson, B. A. S. & Misconi, N. Y. *Bull. AAS* **10**, 653 (1978).

Deposition rate and seasonal variations in precipitation of cosmogenic ^{10}Be

G. M. Raisbeck & F. Yiou

Laboratoire René Bernas, Centre de Spectrométrie Nucléaire et de Spectrométrie de Masse - Bât. 108, 91406 Orsay, France

M. Fruneau, J. M. Loiseaux, M. Lieuvin & J. C. Ravel

Institut des Sciences Nucléaires, 38026 Grenoble, France

The measurement of ^{10}Be using nuclear accelerators promises to widen the applications of this cosmogenic isotope considerably¹⁻³. A parameter of fundamental importance in such applications is the production rate of this nuclide in the atmosphere. This production rate has previously been estimated by two methods: (1) by measuring the concentration of ^{10}Be in marine sediments having known accumulation rates; (2) by using model calculations of cosmic ray interaction rates, combined with estimated cross-sections for production of ^{10}Be . These two methods have led to an estimated global average ^{10}Be production rate of 0.015–0.018 atoms $\text{cm}^{-2} \text{s}^{-1}$ (refs 4, 5 and F. Guichard, unpublished work). The increased sensitivity available with the accelerator technique now makes it feasible to measure directly ^{10}Be deposition rates at the Earth's surface. We report here the results of such a measurement, which lead to an estimated global average production rate more than double the previous estimates. We also give some initial results on the seasonal variation of ^{10}Be concentration in precipitation, and comment on the possibility that such variations might permit the determination of ancient accumulation rates of polar ice.

The ^{10}Be for our measurements was collected in a child's plastic swimming pool (area 2.5 m^2) exposed on the roof of the Laboratoire René Bernas ($\sim 49^\circ \text{N}$) between 3 April 1978 and 6 April 1979. Initially 10 mg of ^9Be and several litres of water were added to the swimming pool. At the end of the exposure period the swimming pool contained ~ 700 l. A known fraction (10 l) of this volume was recovered and analysed for ^9Be by flameless atomic absorption spectrometry. This allowed us to determine the fraction of the initial ^9Be (and thus deposited ^{10}Be) which was recovered. This value agreed very well with the fractional volume of water recovered, showing that essentially all the Be had remained in solution. (This somewhat surprising result can probably be explained by the pH of the precipitation, which was ~ 5.5 at the time of recovery). As well as determining the yearly average by a single measurement, the above method of collection includes any contribution from 'dry' deposition.

Additional ^9Be (4 mg) was added to the 10-l sample which was then passed through a Chelex ion exchange column on which the Be was retained. The Be was then eluted in 1.5M HCl, and purified by a series of solvent extractions⁶. The Be was finally transformed into a sintered BeO disk by a process described elsewhere¹.

The measurements on the cyclotron were carried out as described elsewhere². An important improvement, however, was that samples and standards could now be mounted on a rotating source holder, which permitted us to change samples remotely in a matter of seconds, without modifying any of the accelerator parameters. Details of this system will be given elsewhere.

From the measured $^{10}\text{Be}/^9\text{Be}$ ratio, the known amount of ^9Be added and the known fraction of Be extracted from the swimming pool, we can directly calculate the total number of ^{10}Be atoms and the average rate of deposition during the year. The result is 0.055 ± 0.008 atoms $\text{cm}^{-2} \text{s}^{-1}$. The error comes essen-

tially from the uncertainty on the measured $^{10}\text{Be}/^9\text{Be}$ ratio, and on the measured fraction of ^9Be recovered from the swimming pool.

To estimate an average global deposition rate, we first adjust the above value to the yearly average rainfall for latitude $\sim 50^\circ \text{N}$. This correction is based on the assumption that the deposition of ^{10}Be at a given latitude will be approximately proportional to the quantity of precipitation⁷. As the average rainfall for 50°N is estimated⁸ to be 75 cm compared with a value of 66.7 cm at Orsay during our measurement period, the adjusted average deposition rate at 50°N is 0.062 atoms $\text{cm}^{-2} \text{s}^{-1}$. We next apply a factor for the variation of deposition as a function of latitude as given in Fig. 22 of ref. 7. The final result gives a global average value of 0.042 atoms $\text{cm}^{-2} \text{s}^{-1}$.

It is difficult to evaluate the uncertainty in the two correction factors which transformed our basic data into an average global rate. However, we believe that our average global rate is the best estimate of the ^{10}Be production rate currently available, and that it is significantly larger than the estimates referred to earlier. It would be interesting to consider the possible sources of error in the earlier estimates. Note that in the case of estimates based on marine sediments^{4,5}, relatively few samples were involved, and these tended to be biased towards slow accumulation rate areas (to maximise the concentration of ^{10}Be , thus giving adequate activities for the classical counting technique). It is uncertain whether such sediments can be considered representative of the entire ocean floor. In fact, Tanaka and Inoue⁹ have measured in several sediment cores ^{10}Be deposition rates comparable to those deduced above. In a rapidly (1.13 cm per 10^3 yr) accumulating sediment at 45°N , we have measured a ^{10}Be deposition rate of 0.10 atoms $\text{cm}^{-2} \text{s}^{-1}$ (ref. 10). Thus, when a larger and more representative series of sediment results is available, it may well be consistent with our above estimate. Of course, one must also remember that sediment results refer to an integrated production rate over a considerable (and variable) period of time in history, when all the parameters influencing the production (geomagnetic field, solar modulation, primary cosmic ray intensity) may not have been identical to the present.

With regard to estimates based on cosmic ray interaction rates (ref. 4 and F. Guichard, unpublished work) there is a basic problem in choosing appropriate cross-sections. The most important particles in cosmogenic nuclide production in the atmosphere are neutrons. As very few high-energy neutron induced cross-sections are available, values based on proton-induced reactions are usually substituted. As we have pointed out¹¹, in the particular case of ^{10}Be formed from ^{16}O and ^{14}N , it is quite possible that the neutron induced reactions have larger cross-sections than those induced by protons. We therefore would not be surprised if calculations based on proton cross-sections were underestimated.

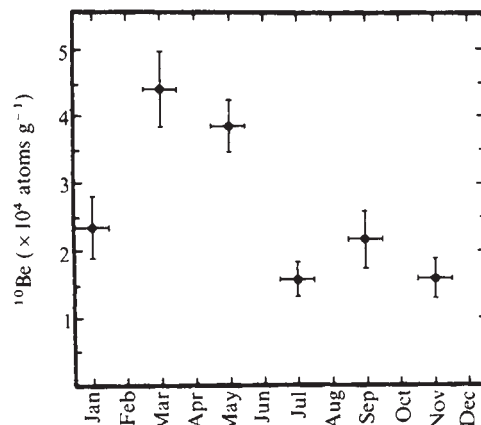


Fig. 1 ^{10}Be concentration in monthly precipitation samples collected in France during 1966.

In addition to the above determination of the average ^{10}Be deposition rate, we have begun a series of measurements on monthly precipitation samples collected over a 10-yr interval by the Commissariat à l'Energie Atomique (France). Our studies are designed to look for seasonal variations, and eventually, effects due to varying solar modulation. The samples, (30–150 l) were collected at Paris, Orsay, Verdun and Bordeaux using plastic funnels having an area of 0.57 m^2 . The precipitation was acidified and reduced to a volume of 15 ml by evaporation. After γ -ray analysis, the samples from the four locations were combined and stored in plastic bottles. We added 4 mg of ^9Be to each of these combined samples, which were treated by the solvent extraction process, made into pellets, and measured on the cyclotron as described above.

The results for six of these samples, collected in 1966, are shown in Fig. 1. There is a sharp increase in the ^{10}Be deposition rate during the spring. This is not unexpected, as it corresponds to the period in the year when there is a major transfer of stratospheric air (where $\sim 70\%$ of the ^{10}Be is formed⁷) into the troposphere¹². It is interesting to consider the possibility that such a seasonal variation might provide a technique for determining accumulation rates in polar ice cores. Our present sensitivity with the cyclotron ($\sim 10^7$ atoms) has the potential for measuring monthly time resolution with currently available ice cores, and we are preparing a series of samples to test this possibility. However, note that the variations are expected to be smaller in polar regions because the predicted stratospheric component is much less at high latitudes⁷.

Finally, while we do not yet have a full year's data for 1966, we can compare the average ^{10}Be concentration in precipitation for the six months analysed (2.33×10^4 atoms g^{-1}) with that for the 1978–79 period (calculated from measured ^{10}Be deposition and the known precipitation rate during that period to be 2.49×10^4 atoms g^{-1}). The agreement is sufficiently good to give us confidence that this is indeed a fairly typical value for France. For a more precise global average deposition rate, similar measurements are needed at several different latitudes.

We thank J. Lestringuez for technical assistance and J. Hémon for the precipitation samples collected by the Laboratoire de Métrologie sur l'environnement, C.E.A. (France).

Received 17 July; accepted 28 September 1979.

1. Raisbeck, G. M., Yiou, F., Fruneau, M. & Loiseaux, J. M. *Science* **202**, 215–217 (1978).
2. Raisbeck, G. M., Yiou, F., Fruneau, M., Lieuvain, M. & Loiseaux, J. M. *Nature* **275**, 731–733 (1978); *Earth planet. Sci. Lett.* **43**, 237–240 (1979).
3. Turekian, K. K. *et al. Geophys. Res. Lett.* **6**, 417–420 (1979).
4. Amin, B. S., Lal, D. & Somayajulu, B. L. K. *Geochim. cosmochim. Acta* **39**, 1187–1192 (1975).
5. Somayajulu, B. L. K. *Geochim. cosmochim. Acta* **41**, 909–913 (1977).
6. Merrill, J. R., Honda, M. & Arnold, J. R. *Analyt. Chem.* **32**, 1420–1426 (1960).
7. Lal, D. & Peters, B. in *Handbook der Physik* Vol. 46/2 (ed. Sitte, K.) 551–612 (Springer, Berlin, 1967).
8. Möller, F. *Petermanns Geogr. Mitt.* **95**, 1–7 (1951).
9. Tanaka, S. & Inoue, T. *Earth planet. Sci. Lett.* (submitted).
10. Raisbeck, G. M. *et al. Geophys. Res. Lett.* **6**, 717 (1979).
11. Yiou, F. & Raisbeck, G. M. *Phys. Rev. Lett.* **29**, 372–375 (1972); *Phys. Rev.* **C9**, 1385–1395 (1974).
12. Reiter, E. R. *Rev. Geophys. Space Phys.* **13**, 459–474 (1975).

Unified yes, renormalised maybe

S. A. Bludman

Department of Physics, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Several recent reports^{1–3} have suggested that recent neutrino and polarised electron scattering experiments are not particular tests of the 'standard theory' of weak interactions, but only establish the strength and form of the weak neutral current

$$J_\lambda^w = J_\lambda^3 - \sin^2 \theta_w J_\lambda^{\text{em}} \quad (1)$$

appearing in the effective weak lagrangian

$$\mathcal{L}^{\text{eff}} = \frac{4G}{\sqrt{2}} [J_\lambda^w J_\lambda^w + J_\lambda^+ J_\lambda^- + J_\lambda^- J_\lambda^+] \quad (2)$$

along with the classical charged-current ($J^\pm$) interactions. Whether or not weak vector mesons exist, the current experiments confirm the existence of an effective lagrangian (2) containing a weak neutral current (1) with the one parameter $\sin^2 \theta_w$. This letter shows that this already establishes some unification of electron and weak interactions, so that the question is no longer "Is unification really true?"³, but rather "Is the theory $\text{SU}_2 \times \text{U}_1$ symmetric and renormalisable?" It also shows that, although weak charged currents must show structure¹ below 160 GeV, the weak neutral currents can be structureless, that is $m_\pi = \infty$ is compatible with the observed current (1).

The first gauge theory of chiral weak interactions⁵ had $\sin^2 \theta_w = 0$ so that $\mathcal{L}^{\text{eff}} = (4G/\sqrt{2}) \mathbf{J} \cdot \mathbf{J}$ was $\text{SU}_2(w)$ symmetric. This theory ignored electromagnetism and predicted for the first time weak neutral currents of the same strength and form as the weak charged currents. (While this theory was a local gauge theory involving a triplet of vector mesons $w^\pm$ and w^0 , the global $\text{SU}_2(w)$ suffices for present purposes.) Glashow⁴ coupled in electromagnetism, extending the theory to $\text{SU}_2 \times \text{U}_1$, and first proposed the form (1). The history of neutral currents and the local gauge theory of weak interactions after ref. 5 is documented in refs 6, 7. The suppression of strangeness-changing neutral currents⁸ and the proof of renormalisability^{9–11} were most important. Bludman's and Glashow's ideals were early

emphasised by Gell-Mann¹² and formed the basis for his algebra of currents and charges. Bjorken has shown³ that these $\sin^2 \theta_w J_\lambda^{\text{em}}$ terms in the weak neutral current originate in any large electromagnetic braking of the $\text{SU}_2(w)$ invariance such as an intrinsic neutrino form factor much larger than that induced by charged currents¹³. This symmetry-breaking term arises in any theory incorporating γ - w_0 mixing², but does not require the existence of weak vector mesons and certainly not the particular (Higgs) mechanism for symmetry breaking of the 'standard' theory.

The author was one of the first¹⁴ and most consistent proponents of local gauge theories and to appreciate the theoretical advantages of a renormalisable theory of weak interactions. Renormalisability makes it possible to consider higher order weak processes like the $K_L - K_S$ mass difference and radiative corrections to β - and μ -decay. Certainly the suggestion^{15,16} of such a renormalisable theory, together with the development of neutrino accelerator facilities, helped stimulate the discovery in 1973 of the predicted⁵ neutral currents. Nevertheless, the present experiments confirm only the strength and form of the proposed neutral currents^{4,5}; experiments searching for $W^\pm$, Z^0 and Higgs mesons are needed to test the standard theory^{15,16} of weak interactions. At present, spontaneous symmetry-breaking by Higgs mesons apparently introduces as many problems as it solves, and a future dynamical theory may not involve the present theory of the Higgs mechanism at all.

Indeed, Bjorken shows¹ that the charged-current scattering amplitude must be of the form

$$G(q^2) = G(1 + \frac{q^2}{\mu_w^2} + \dots)$$

with μ_w^{-2} a charged radius bounded from below or μ_w^2 a mean mass bounded from above by

$$\mu_w \leq \frac{e}{(4G\sqrt{2})^{1/2}} \frac{(1 - Z_3)^{1/2}}{\sin^2 \theta_w} = (37.4 \text{ GeV}) \frac{\sin \theta_1}{\sin^2 \theta_w} \quad (3)$$

Here $\sin^2 \theta_w$ is defined by the neutrino charged-radius spectral function and Z_3 is the charge renormalisation induced by intermediate states ('weak quanta') dominating the weak forces. Since $0 \leq Z_3 \leq 1$, we write $Z_3 \equiv \cos^2 \theta_1$. With $\sin^2 \theta_w \approx 0.23 \pm 0.02$, equation (3) shows that charge current weak inter-

actions must show structure somewhere below 160 GeV, whether or not intermediate vector mesons exist. If $W^\pm$ mesons exist, equation (3) becomes an upper limit on their mass, $\mu_w = (160 \text{ GeV}) \sin \theta_1 \leq 160 \text{ GeV}$. We now show that there is a lower limit (equation (6)) on m_z , the mass of the Z^0 . Following Hung and Sakurai², consider a phenomenological $\gamma - w_0$ mixing

$$\mathcal{L}^{\text{mix}} = -\frac{\lambda}{4} (\tilde{F}_{\mu\nu} w_0^\mu + w_{\mu\nu}^0 \tilde{F}^{\mu\nu}), \quad (4)$$

which may be a manifestation of a large intrinsic neutrino charge form factor or may originate in quark or lepton vacuum polarisation loops. $\tilde{F}_{\mu\nu}$ is the original unmixed massless photon field. Since the interaction (equation (4)) is electromagnetically gauge invariant, the photon remains massless after mixing, but the W^0 mass is raised to

$$m_z^2 = \frac{m_w^2}{1 - \lambda^2}$$

As this requires $\lambda^2 \leq 1$ we can write $\lambda^2 \equiv \sin^2 \theta_2$. After mixing, the $\gamma - W_0$ part of the effective lagrangian is

$$\mathcal{L}^{\text{eff}} = \frac{1}{2} \left\{ e^2 J_\lambda^{\text{em}} \frac{1}{q^2} J_\lambda^{\text{em}} + \frac{g^2}{m_w^2} J_\lambda^w \frac{m_z^2}{q^2 + m_z^2} J_\lambda^w \right\} \quad (15)$$

where J_λ^w is given by equation (1) with

$$\sin^2 \theta_w \equiv \frac{e}{g} \lambda \equiv \frac{e}{g} \sin \theta_2$$

Like Bjorken's, this effective lagrangian depends on two parameters, $\sin^2 \theta_w$ and $\sin \theta_1$ or $\sin \theta_2$. (The original $SU_2(w)$ theory predicting neutral currents of universal coupling strength G is recovered if $e/g = 0$ or $\lambda = 0$.)

In terms of θ_2 ,

$$m_z = \frac{m_w}{\cos \theta_2} \quad (6)$$

$$m_w = \frac{g}{(4G\sqrt{2})^{1/2}} = (37.4 \text{ GeV}) \frac{g}{e} = (160 \text{ GeV}) \sin \theta_2 \quad (7)$$

While $W^\pm$ vector mesons, or at least Bjorken's 'virtual quanta', must exist below 160 GeV, Z^0 could be infinite in mass. In such a case, there would be only two charged vector mesons of mass $m_w = 160 \text{ GeV}$, and the neutral electro-weak interactions would retain their low energy form at all energies: electromagnetic and weak neutral interactions would be simply of infinite and zero range respectively.

The present generation of experiments establishes the existence of the neutral current (1) with $\sin^2 \theta_w \approx 0.23 \pm 0.02$ and $m_w > 30 \text{ GeV}$. Since this makes $g/e > 0.8$ or the mixing parameter $\lambda \equiv \sin \theta_2 > 0.2$, these experiments demonstrate large electromagnetic breaking⁴ of the original⁵ $SU_2(w)$ symmetry, or a particle mixing theory that is unified on the tree level of approximation. (The magnitude observed for $\sin^2 \theta_w$ may suggest the importance of higher order effects, which would require a renormalisable theory for their calculation.)

At $q^2 \ll m_z^2$ the effective lagrangian (5) reproduces the ordinary electromagnetic interactions plus the observed weak currents of universal strength $g^2/m_w^2 = 4G\sqrt{2}$. At $q^2 \gg m_z^2$

$$\mathcal{L}^{\text{eff}} \approx \frac{1}{2 \cos^2 \theta_2} \left\{ e^2 J^\gamma J^\gamma + (e^2 + g^2 - 2eg \sin \theta_2) J^3 J^3 + 2e(e - g \sin \theta_2) J_\lambda^3 J_\lambda^3 \right\} \quad (8)$$

Hung and Sakurai² show the special choice of mixing parameter

$$\lambda = e/g \quad (9)$$

or $\sin \theta_2 = \sin \theta_w$ makes the $\gamma - w_0$ theory asymptotically $SU_2 \times U_1$ symmetric and tree unitary¹⁷⁻²⁰. It gives the $w^\pm$ vector mesons gyromagnetic ratio $g = 2$ and predicts

$$m_w = \frac{37.4 \text{ GeV}}{\sin \theta_w} = 80 \text{ GeV} \quad (10)$$

$$m_z = \frac{m_w}{\cos \theta_w} = 90 \text{ GeV} \quad (11)$$

Whether equations (9)–(11) turn out to be satisfied or not, present experiments establish unification in the tree level of approximation. Neither the two-parameter theory we are proposing nor the standard $SU_2 \times U_1$ theory is yet unified in the sense of containing one coupling constant in a simple group structure. The condition (9), which Hung and Sakurai called the 'unification condition', might therefore more properly be called the 'renormalisability condition': it guarantees good high-energy behaviour.

The mass relations (10), (11) are those derived in the 'standard theory' from a local $SU_2 \times U_1$ renormalisable theory with symmetry spontaneously broken by the simplest Higgs mechanism. Future experiments may indeed demonstrate the existence of $W^\pm$ and Z^0 mesons of masses (10), (11) and even possibly Higgs scalar mesons. Until then, however, we can only say that present experiments prove the existence of weak neutral currents of the strength originally predicted⁵, with strong electromagnetic breaking⁴ of the original $SU_2(w)$ symmetry and leave open the possibility $m_w = 160 \text{ GeV}$, $m_z = \infty$.

I thank S. Gasiorowicz, M. Gell-Mann for stimulating discussions and others at the Aspen Center of Physics where I have enjoyed many summers. This work was supported in part by the US Department of Energy under contract EY-76-CI-02-3071.

Received 15 August; accepted 25 September 1979.

1. Bjorken, J. D. *Phys. Rev.* **D19**, 335–346 (1979).
2. Hung, P. Q., & Sakurai, D. J. *Nucl. Phys.* **B143**, 81–113 (1978).
3. Dombey, N. *Nature* **279**, 675–677 (1979).
4. Glashow, S. *Nucl. Phys.* **22**, 579–588 (1961).
5. Bludman, S. A. *Nuovo Cimento, Ser. 10* **IX**, 433–445 (1958).
6. Veltman, M. *Proc. Vth Intl. Symp. on Electron and Photon Interactions at High Energies* (North-Holland, Amsterdam, 1974).
7. Sakurai, J. J. *Current Trends in the Theory of Fields* (A.I.P. Proc. No. 48, 1978); *III Int. Symp. on High Energy Physics with Polarized Beams and Polarized Targets* (A.I.P. Proc., 1978).
8. Glashow, S. L., Iliopoulos, J. & Maiani, L. *Phys. Rev.* **D2**, 1285 (1970).
9. 't Hooft, G. *Nucl. Phys.* **B33**, 173–199 (1971); **B35**, 167–188 (1971).
10. Lee, B. W. & Zinn Justin, J. *Phys. Rev.* **D5**, 3121–3137, 3137–3155; 3155–3160 (1972).
11. 't Hooft, G. & Veltman, M. *Nucl. Phys.* **B44**, 189–213 (1972).
12. Gell-Mann, M. *Proc. Int. Conf. on High Energy Physics, Rochester* (1960).
13. Bludman, S. A. & Cheng, W. K. *Phys. Rev.* **B136**, 1787–1790 (1964).
14. Bludman, S. A. *Phys. Rev.* **100**, 372–375 (1955).
15. Weinberg, S. *Phys. Rev. Lett.* **19**, 1264–1266 (1967).
16. Salam, S. *Elementary Particle Theory* (ed. Svartholm, N.) (Almqvist and Wiksells, Stockholm, 1968).
17. Weinberg, S. *Phys. Rev. Lett.* **27**, 1688–1691 (1971).
18. Bell, J. S. *Nucl. Phys.* **B60**, 427–436 (1973).
19. Llewellyn Smith, C. H. *Phys. Lett.* **46B**, 233–236 (1973).
20. Cornwell, J. M., Levin, D. & Tiktopoulos, G. *Phys. Rev. Lett.* **30**, 1268–1270 (1973).

Electrocatalytic oxygen evolution on reactively sputtered electrochromic iridium oxide films

G. Beni, L. M. Schiavone, J. L. Shay, W. C. Dautremont-Smith & B. S. Schneider

Bell Laboratories, Holmdel and Murray Hill, New Jersey 07733

Oxygen evolution reactions¹ are critical to the development of efficient energy conversion and energy storage devices. For example, power losses at the oxygen electrodes of commercial water electrolyzers² and H_2 - O_2 fuel cells limit their efficiencies to values well below those expected theoretically. The power loss of these devices is $\geq 15\%$ even at low current densities, and much higher in practical operating conditions. We report here the preparation and the oxygen evolution reaction (OER) electrocatalytic properties of iridium oxide films deposited by reactively sputtering iridium in a humidified oxygen discharge. We find that these sputtered iridium oxide films (SIROFs) have catalytic properties far superior to the best known catalysts for oxygen evolution in acidic aqueous electrolytes at room temperature.

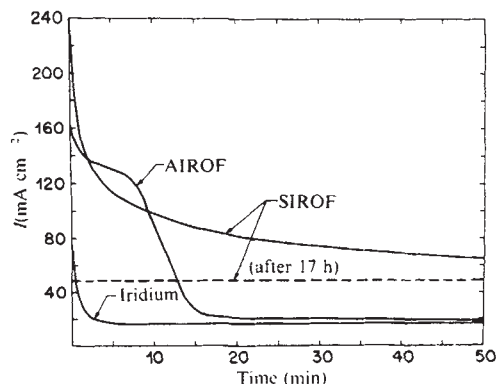


Fig. 1 Oxygen evolution reaction (OER) currents at 1.85 V against RHE as a function of time for anodic iridium oxide films (AIROF), sputtered iridium oxide films (SIROF), and for pure iridium in 0.5 M H_2SO_4 at 25 °C. Despite their high initial current, AIROFs dissolve in the acidic solution so that after ~20 min their OER current is about the same as for bare iridium. In contrast, SIROFs are stable. The dashed line shows the current of SIROFs in the steady state.

Few studies have been aimed at establishing the factors affecting electrocatalysis of O_2 , so that we lack the information necessary for a theoretical approach to the search for better O_2 electrocatalysts. Semi-empirically, it has become accepted¹ that a good OER catalyst must have a relatively high electronic conductivity and a high affinity for adsorbed OH^- intermediates. The electrode must not corrode; hence much electrocatalysis research has been restricted to the noble metals and their alloys. A number of recent studies³⁻⁶, however, have pointed out that the link among different electrodes known to be good electrocatalysts for OER (for example, Ir, Ru, Ni) seems to be a relatively thick oxide film which is present on their surfaces. This thick oxide film is clearly different from the more common thin oxide film found on the surface of many metals. In fact these thin films are known to inhibit the rate of many chemical reactions, including the OER. This is why much research ostensibly aimed at solving the problem of electrocatalysis focuses on surfaces devoid of oxides, that is, 'clean' surfaces. In contrast, for 'real' catalysts, thick oxide films ($\geq 1,000 \text{ \AA}$) bring about an improved OER.

Experiments show that these thick oxide films display unusual electrochromic effects. Most remarkable is the electrochromism⁷⁻¹⁰ of anodic iridium oxide films (AIROFs). Colour-switching times are comparable to those of liquid crystals. AIROF electrochromism takes place at ~1 V (RHE) in 0.5 M H_2SO_4 , and is thus a precursor of the OER which occurs at ~1.5 V (RHE). Although the relationship between electrochromism and OER catalysis is not yet completely understood, it is possible that a higher electronic conductivity and increased OH^- content in the coloured state favours the OER. In fact, AIROFs show^{3-6,11} good electrocatalytic activity for the OER. A comparison⁵ with the performance of the bare iridium electrode indicated that currents obtained for AIROFs on Ir were larger than currents obtained at the same potential on bare Ir.

Although AIROFs have higher OER rates than Ir, they dissolve at significant rates at low voltages (1.4–1.6 V against RHE), and very rapidly at voltages above 1.6 (RHE) in acidic solutions, rendering them of no use as practical catalysts. This initially high catalytic activity and subsequent degradation of AIROFs is illustrated in Fig. 1. After ~20 min the AIROF is totally dissolved and the exposed surface is bare iridium. Thus, in AIROFs, there seems to be a competition between catalytic activity and stability. The high catalytic activity and instability of AIROFs is consistent with the existence of metastable states of intermediate oxidation.

In an attempt to stabilise these metastable states in iridium oxide we reactively sputtered iridium in a humidified oxygen

discharge. In the sputter deposition of a thin film, highly energetic species in the vapour phase impinge onto a cold substrate. The rapid quenching of the deposited material tends to stabilise structures that would normally be unstable at room temperature.

SIROFs were deposited on a variety of substrates using an iridium target in a Cooke model C-70-6-4B rf sputtering system. The substrates were washed in a detergent bath, rinsed in distilled water, ultrasonically cleaned in isopropyl alcohol, and vapour-degreased with isopropyl alcohol. They were then placed on a water-cooled J-arm substrate table in the sputtering chamber, which was subsequently evacuated to a pressure of $\sim 2 \times 10^{-7}$ torr. Oxygen at a pressure of 17 p.s.i. saturated with water at room temperature, was admitted into the chamber to a pressure of $\sim 30\text{-}\mu\text{mHg}$. After a few minutes, the plasma was ignited and the pressure was reduced to $\sim 8\text{-}\mu\text{mHg}$. Deposition times were ~1 h, typically at an r.f. power of 75 W to a 2"-diameter iridium target corresponding to a target d.c. bias of ~700 V. The metal substrate did not affect the SIROF composition; for example, in the case of tantalum substrates, Auger measurements showed no evidence for any Ta at or near the SIROF surface.

We show elsewhere¹² that the SIROFs have many of the desirable electrochromic properties of AIROFs, including the fast electrochromic response times. The voltammograms for SIROFs are similar to those for AIROFs but differ in detail. In particular, the electrochromic peak for the SIROF occurs at ~0.80 V (RHE) rather than at ~0.95 V (RHE). Both voltammograms are highly symmetric, resulting in excellent stability when operated in the electrochromic range of potentials. We show here that SIROFs (in contrast to AIROFs) are also stable when operated as catalysts in the OER region of potentials, and exhibit a much higher catalytic activity for the OER than bare iridium. Long-term stability tests have not been conducted yet. However, as no degradation has been obtained after ~20 h of operation, SIROFs are at least 2 orders of magnitude more stable than AIROFs.

Figure 1 shows the current of OER for SIROF, AIROF and Ir at 1.85 V (RHE). All currents decay with time until they reach a steady state value (after a few minutes for iridium and after a few hours for SIROF). As noted, AIROFs dissolve rapidly (~20 min). The dashed line shows the steady-state current for SIROFs recorded after ~17 h. The steady-state current is ~3 times as large as the current for iridium, hitherto the best catalyst for OER in acidic solutions.

Tafel plots for OER on SIROFs can be obtained from the steady-state currents recorded after ~17 h at various potentials with no prepolarisation (Fig. 2). Tafel plots for AIROFs have been reported⁵. These plots, however, are practically meaningless as a measure of the AIROF activity because: (1) the currents were arbitrarily recorded 10 min after the initial application of

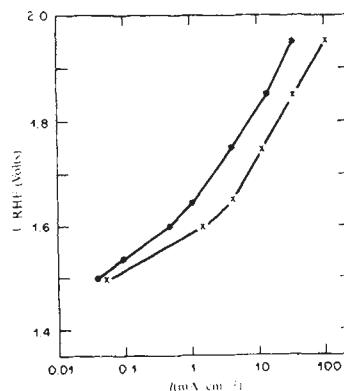


Fig. 2 Tafel slopes for Ir (●) and for SIROFs (×) in 0.5 M H_2SO_4 . The data are obtained from currents recorded in the steady state (after ~17 h) with no prepolarisation. Temperature, 25 °C.

the potential to the AIROF electrode; and (2) AIROFs corrode so that the currents recorded are not OER currents but include dissolution currents. At high voltages the slope is essentially equal to that of Ir and other precious metals¹³. An independent measure of the catalytic activity of the material can be obtained by plotting the initial current, after subtracting out the capacitive component. Although the details of this analysis will be reported elsewhere (G.B., L.M.S., J.L.S. and W.C.D.-S., in preparation), both steady state and transient measurements clearly indicate the superior catalytic activity of SIROFs.

In general, oxygen evolved on a metal electrode reacts with the metal itself to form an oxide. Thus, the potential at which oxygen is free to leave the electrode is determined by the potential of the metal/metal-oxide couple, or if there is more than one oxide, by the potential of the lower-metal-oxide/higher-metal-oxide couple. Empirically, it has been found¹ that the lower these potentials, the higher the catalytic activity.

Our results confirm this trend. In fact, for AIROFs and SIROFs, the lower-metal-oxide/higher-metal-oxide potential coincides with the electrochromic peak of the voltammogram. As this peak occurs at ~ 0.80 V (RHE) in SIROFs and at ~ 0.95 V (RHE) in AIROFs, the higher catalytic activity of the SIROF is consistent with the lower potential of its intermediate oxidation states. Moreover, it is likely that the reactive sputtering procedure 'freezes in' these metastable states of intermediate oxidation, resulting in high stability. Thus, in SIROFs, unlike in AIROFs, no trade-off exists between catalytic activity and stability.

We have shown that stable and efficient electrocatalysts for the oxygen electrode can be fabricated by reactively sputtering iridium in a humidified ambient atmosphere and depositing the sputtered films on substrates of non-precious metals such as tantalum. Also the catalytic activity of the SIROFs is better than that of pure iridium, and this material is stable against dissolution in acidic solutions. As this comparison is based on geometric areas, the 'intrinsic' catalytic activity of SIROFs is still unknown. Measurements of the real SIROF area are in progress. Preliminary results (G.B., L.M.S., J.L.S. and W.C.D.-S., in preparation) in alkaline solutions show that steady-state OER currents for SIROFs far exceed the values obtained with the preferred catalysts for alkaline electrolyzers¹⁴⁻¹⁸. For example, whereas NiCo_2O_4 , at 1.69 V against RHE has an OER current¹⁹ of 50 mA cm^{-2} at room temperature, SIROFs have OER currents in excess of 1 A cm^{-2} after 17 h of operation (G.B., L.M.S., J.L.S. and W.C.D.-S., in preparation). Thus, although obviously not optimised, SIROFs seem to be very promising OER catalysts.

We thank A. M. Glass and D. H. Olson for the use of their sputtering apparatus, E. Hu and P. Grabbe for the Auger measurements, and A. Heller, C. E. Rice and R. J. H. Voorhoeve for useful comments.

Received 26 June; accepted 28 September 1979.

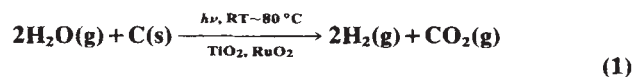
- Hoare, J. P. *The Electrochemistry of Oxygen* (Interscience, New York, 1968).
- Extended Abstr. Spring Meet. electrochem. Soc. (Seattle, Washington, 1978).
- Rand, D. A. J. & Woods, R. J. *Electroanal. Chem.* **55**, 375 (1974).
- Buckley, D. N. & Burke, L. D. *JCS Faraday I* **71**, 1447 (1975).
- See e.g. Gottesfeld, S. & Srinivasan, S. *J. electroanal. Chem.* **84**, 117 (1977).
- Horkans, G. J. & Shafer, M. W. *J. electrochem. Soc.* **124**, 1202 (1977).
- Gottesfeld, S., McIntyre, J. D. E., Beni, G. & Shay, J. L. *Appl. phys. Lett.* **33**, 208 (1978).
- Beni, G. & Shay, J. L. *Appl. phys. Lett.* **33**, 567 (1978); *Phys. Rev. B* (in the press).
- Shay, J. L., Beni, G. & Schiavone, L. M. *Appl. phys. Lett.* **33**, 942 (1978).
- Gottesfeld, S. & McIntyre, J. D. E. *J. electrochem. Soc.* **126**, 742 (1979).
- Buckley, D. N. & Burke, L. A. *JCS Faraday I* **72**, 2431 (1976).
- Schiavone, L. M., Dautremont-Smith, W. C., Beni, G. & Shay, J. L. *Appl. phys. Lett.* **35**, 823 (1979).
- See e.g. Bockris, J. O'M. & Reddy, A. K. N. *Modern Electrochemistry* (Plenum, New York, 1970).
- Nidola, A., Spaziante, P. M. & Giuffrè, L. *Extended Abstr. Spring Meet. electrochem. Soc.* 1202 (Seattle, Washington, 1978).
- Bergner, D. & Kohlhaas, R. *German patent no.* 2,150,039.
- DeNora, O., Nidola, A. & Trisoglio, G. *Italian patent no.* 1,398,211.
- Saito, S., Ohe, K. & Kawai, Y. *Japanese patent no.* 7,798,684.
- Kolb, J. M. & O'leary, K. J. *German patent no.* 2,331,949 (1974).
- Tseung, A. C. C. & Jassem, S. *Electrochim. Acta* **22**, 31 (1977).

Hydrogen evolution from water using solid carbon and light energy

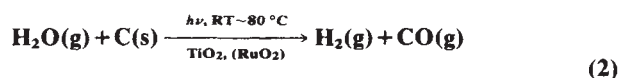
Tomoji Kawai & Tadayoshi Sakata

Institute for Molecular Science, Myodaiji, Okazaki 444, Japan

Using solar energy to decompose water and produce hydrogen for use in hydrogen energy systems has attracted much attention. Some semiconductors (such as TiO_2 and SrTiO_3) can decompose water to produce hydrogen and oxygen using the energy¹ of light and the decomposition of water using powdered semiconductors has been reported^{2,3}. One problem with powdered semiconductors is that when H_2O decomposes into H_2 and O_2 in one cell an explosive gas is produced. Furthermore, although Schrauzer and Guth reported² that traces of H_2 and O_2 were detected after the decomposition of water gas on TiO_2 , the reverse reaction will increase simultaneously as product is evolved. Hydrogen evolution stopped after a few hours. Hence the hydrogen or oxygen produced should be converted to more stable gaseous chemical species. The use of solid carbon is an interesting problem in relation to coal gasification and we have now examined the photocatalytic decomposition of water on TiO_2 using solid carbon. We found that reactions (1) and (2)



$$\Delta G^\circ = +63 \text{ kJ mol}^{-1}$$



$$\Delta G^\circ = +92 \text{ kJ mol}^{-1}$$



occur producing hydrogen gas from water vapour and solid carbon when mixed powders of TiO_2 , RuO_2 and active carbon exposed to water vapour at room temperature $\sim 80^\circ\text{C}$ are illuminated. The free energies are increased by 63 and 92 kJ mol^{-1} respectively, utilising light energy.

In our experiments 30 mg of TiO_2 (Katayama Kagaku), 10 mg of RuO_2 (Rare Metallic Co.) and 3 mg of active carbon (Katayama Kagaku) were mixed and rubbed in an agate mortar, and spread inside a Pyrex glass bulb (20 cm^2 sample area). The cell and its side arm containing liquid water were then evacuated and illuminated for 5 h in water vapour by a 500 W high-pressure mercury lamp. Any light having a wavelength longer than 320 nm can pass through a Pyrex glass bulb. The gas evolved was trapped at -197 and -78°C , and was analysed by a mass spectrometer. The gas which was not trapped at liquid N_2 temperature was mainly hydrogen (0.2 torr in 350 ml vessel) and a small amount of carbon monoxide. The gas which was not trapped at -78°C was mainly carbon dioxide ($\text{H}_2 : \text{CO}_2 = 1-1.5$) (Fig. 1). When D_2O was used instead of H_2O , D_2 was detected, proving that the evolved hydrogen had been produced from the decomposition of water. Tests were carried out on the systems, $\text{TiO}_2\text{-H}_2\text{O}$ to examine the effect of impurities, and $\text{SiO}_2\text{-C-H}_2\text{O}$ to confirm that without the TiO_2 photocatalyst no appreciable H_2 , CO or CO_2 evolved.

Direct reaction of carbon (coke) with water using heat energy is known as the water gas reaction (2) which takes place at temperatures above $1,000^\circ\text{C}$. In our photocatalytic system, however, the reaction with water proceeds at lower temperatures, using carbon as the raw material to decompose water and produce hydrogen. This indicates that oxygen (atom or molecule) produced on the surface of the photocatalyst ($\text{TiO}_2\text{-RuO}_2$) has a strong oxidising effect on the carbon.

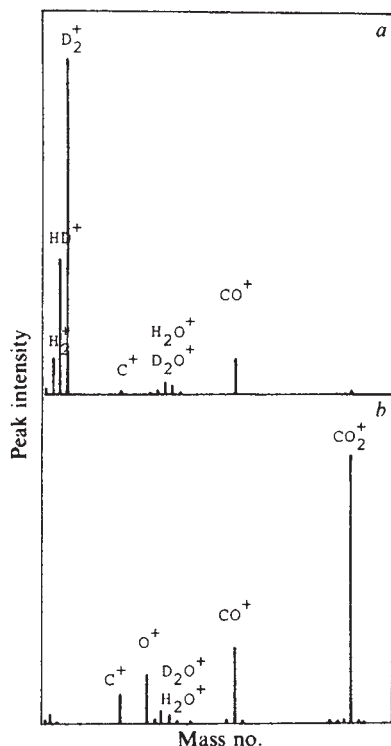


Fig. 1 Mass spectra of the photoproducted gases on $\text{TiO}_2\text{-RuO}_2\text{-C-D}_2\text{O}$ (90%). *a*, Fraction not trapped at -197°C : D_2 , H_2 and small amount of CO ; *b*, fraction not trapped at -78°C : mainly CO_2 . The C^+ , O^+ and CO^+ in *b* are fragments of CO_2^+ . H_2O^+ and D_2O^+ are due to background gas and incompleteness of the trapping of water. Pressure of the mass chamber during measurements are 2×10^{-7} torr. 'No hydrogen evolution' means that no D_2 signal was noted when the sample gas was introduced into the mass spectrometer after the photolysis using D_2O . The amount of evolved H_2 and CO depended on how well the catalyst powders were rubbed in an agate mortar. Therefore, the average values obtained after five experiments were considered here.

As TiO_2 powder is regarded as a microcell for the photoredox reaction, water will be oxidised to form oxygen which reacts with carbon to form CO and CO_2 , whereas at the reduction site, hydrogen will be formed. Carbon by its own oxidation acts as a reducing reagent. RuO_2 is one of the best electrode materials for oxygen evolution with the lowest overpotential⁴. Hence it may act as the oxidation site at which carbon forms CO and CO_2 . In fact, CO_2 formation was suppressed when the RuO_2 catalyst was not added to the system. Only traces of hydrogen molecule could be detected when oxidised TiO_2 photocatalyst alone was used. The addition of RuO_2 and solid carbon markedly increased the hydrogen evolution. Although absolute quantum yield of the reaction has not yet been obtained, the relative quantum efficiency of H_2 evolution in the $\text{TiO}_2\text{-RuO}_2$, C , H_2O system increased by at least 10^2 times that from the $\text{TiO}_2\text{-H}_2\text{O}$ system we tested. Consequently, although the increased energy per reactions (1) and (2) is less than that of the decomposition of water into H_2 and O_2 , the total energy efficiency for the incident light is much larger in our system. We have examined the effect of several kinds of metals as catalyst (Ru , Ni and Pt) with TiO_2 . Although the evolved hydrogen is increased $\sim 7\text{--}15$ times in the first stage of the reaction, this would be due to the oxidation of metal itself by the oxygen produced from H_2O , assisting hydrogen evolution. Hence the system combined with metal-oxide catalyst is catalytically regenerative. This system remained catalytically active for more than 7 days illumination. When the temperature was raised from room temperature to $60\text{--}80^\circ\text{C}$, the rate of CO and CO_2 formation increased, probably because a lot of energy is required to split the C-C bond of active carbon to form CO or CO_2 . Therefore the use of solar energy as a combination of light energy and heat energy will be more useful.

In the photo-driven ammonia synthesis from N_2 and H_2O (ref. 2) and CO_2 reduction by H_2O (refs 5, 6), hydrogen produced from water was used, whereas the oxygen evolved reacted with carbon, and in our system gaseous hydrogen is produced. Note also that during the reaction, hydrocarbons were produced by reactions among photo-products or solid carbon directly.

As the Earth contains a lot of carbon in the form of coal, this method may be a possibility for coal gasification and hydrogen evolution from water, accompanied by storage of solar energy.

Received 6 July; accepted 18 September 1979.

1. Maruska, H. P. & Ghosh, A. K. *Solar Energy* **20**, 443 (1978).
2. Schrauzer, G. N. & Guth, T. D. *J. Am. chem. Soc.* **99**, 7189 (1977).
3. Krasnovskii, A. A. & Brin, G. P. *Dokl. Akad. Nauk SSSR* **168**, 1100 (1966).
4. Morita, M., Iwakura, C. & Tamura, H. *Electrochim. Acta* **23**, 331 (1978).
5. Hemminger, J. C., Carr, R. & Somorjai, G. A. *Chem. phys. Lett.* **57**, 100 (1978).
6. Inoue, T., Fujishima, A., Konishi, S. & Honda, K. *Nature* **277**, 637 (1979).

Phosphorylation of adenosine in aqueous solution by electric discharges

Y. Yamagata, T. Matsukawa, T. Mohri & K. Inomata*

Department of Physics and * Department of Chemistry, Faculty of Science, Kanazawa University, Marunouchi, Kanazawa 920, Japan

Dehydration reactions involving condensing reagents in aqueous solution¹ have been studied as models of chemical evolution, and one such reagent has been found to be produced in the supposed primitive Earth conditions². We assumed that the dehydration condensation in aqueous solution could occur if the condensing reagents, which appear to be produced by electric discharges in the gas phase, could be carried to the aqueous solution through the recycling of water washing the wall of the vessel. We present here an experimental study of the dehydration condensation between phosphate and adenosine in aqueous solution using a new discharge apparatus (Fig. 1) which simulates prebiotic chemical evolution. The apparatus was designed so that water recycles in a vessel containing a solution at a relatively low temperature ($\sim 30^\circ\text{C}$), achieved by showering water at 18°C from above the vessel. This is a simulation of the recycling system of water on the Earth, depending on a large difference in temperature between the ocean surface and the sky.

Four parallel discharges simulating lightning were induced by four neon-sign transformers (Matsushita, 15 kV), each connected to a ring co-electrode at the centre of the vessel and one of four electrodes around it. The gaps between the electrodes were 3–4 cm. Nichrome wire of 1.6 mm diameter was used for the electrodes. In each of the experiments the aqueous solution and the gases were prepared in the 5-litre discharge vessel in the conditions shown in Table 1. Adenosine was purified on a DEAE-Sephadex column to eliminate impurities such as AMP and other nucleotides. The solution was kept at about 30°C and stirred with the magnetic stirrer.

The pressure in the vessel changed considerably during the discharge. Figure 2 shows this in runs 1 and 7. The patterns of change were similar in runs 1 to 6, and that of run 8 was to that of run 7 for up to 1 week, thereafter decreasing gradually to 16 cm Hg. The initial increases of the pressure in runs 1–6 are probably due to the decomposition of ammonium bicarbonate. Gas chromatography of the gas component in run 5 [Shimadzu Model GC-6APTF; column: active carbon 3 mm $\times$ 3 m; column temperature $40\text{--}200^\circ\text{C}$; carrier gas He , 40 ml min⁻¹] showed that about 90% of its nitrogen appeared as molecular nitrogen, and 30–40% of its carbon as carbon monoxide and dioxide. In runs 7 and 8 the pressure decreased suddenly at the start of

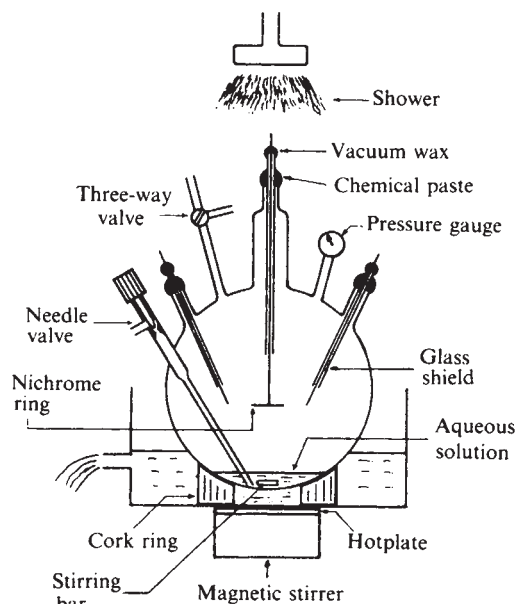


Fig. 1 Five-litre electric discharge apparatus.

discharge, mainly due to consumption of hydrogen. Gas chromatography after a week of discharge showed that the remaining hydrogen constituted only a few per cent of initial level in both cases, but the remainders of the nitrogen and carbon compounds ($\text{CO}_2 + \text{CO}$) were 87% and 74% (18% + 56%) for run 7, respectively, and 85% and 67% (50% + 17%) for run 8 (although these results contain errors of several per cent).

After the discharge had taken place for a week, the solution was applied to a DEAE-Sephadex column (1.5 × 37 cm) and a gradient elution was carried out with 500 ml each of 0.02 M and 0.5 M NH_4HCO_3 (rechromatography was not necessary in runs 7 and 8, because AMP fractions contained only a small quantity of impurities). The fractions expected to contain AMPs were collected, evaporated, and rechromatographed on a Sephadex G-15 column (2.64 × 65 cm) by elution with 0.1 M NH_4HCO_3 (rechromatography was not necessary in runs 7 and 8, because AMP fractions contained only a small quantity of impurities). The fractions of the main peaks corresponding to AMPs were collected and evaporated to remove NH_4HCO_3 , and the residue was subjected to preparative TLC (silica gel) with a solvent system, ethanol ammonia/water, 4:1:1. The extracts from

UV-absorbing bands corresponding to 5'-AMP and 2'(3')-AMP were confirmed to be the expected products on cellulose TLC (solvent system, saturated ammonium sulphate/1M ammonium acetate/isopropyl alcohol, 80:18:2) in which the 2'(3')-AMP component was separated into two nearly equal UV-absorbing spots corresponding to 2'- and 3'-AMP³. Identifications were confirmed by the degradation of the products to adenosine with an application of alkaline phosphatase⁴. Total yields of AMPs are given in Table 1 together with the ratios of the yields of 5'- and 2'(3')-AMP. The yields are relatively high, considering that the yield of phosphorylation is, at most, a few per cent even in the experiments in which a large amount of the condensing agents was used⁵, and the very low yields (0.002–0.02%) of the dicyanamide formation in the $\text{CH}_4\text{-NH}_3\text{-H}_2\text{O}$ mixture irradiated by electrons².

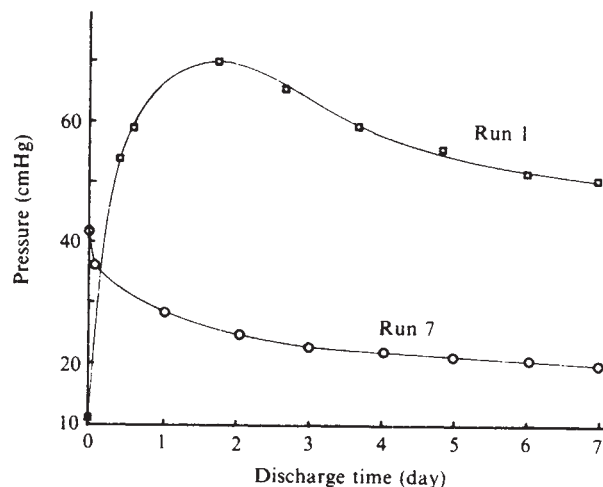


Fig. 2 Pressure change during discharge.

Formation of pyrophosphate was also confirmed in run 8 by detecting phosphate⁶ in the fractions corresponding to pyrophosphate after they were boiled for 8 h or treated with pyrophosphatase⁷. The yield of pyrophosphate was estimated to be about 0.01% of the starting phosphate.

A striking result was the finding that the yield of run 8 was one order of magnitude higher than that of run 7. The control experiment in which no electric discharges were applied was also carried out in the same conditions as run 8. Fifty ml of the solution was pumped out after 2 weeks of the incubation. Apart from adenosine, no UV absorbing substances were detected. This indicates that the yield of AMP would be <0.001% which is the limit of the detection. Discharges in run 8 were continued over 1 week to study the dependence of the yield on the

Table 1 Starting conditions and results of analyses after 1 week of electrical discharge

Starting condition	1	2	3	Run no. 4	5	6	7	8
Aqueous solution (100 ml)								
Adenosine (M)	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02
$(\text{NH}_4)_2\text{HPO}_4$ (M)	0.2	0.2	0.2	0.2	0.2	0.2	—	—
KH_2PO_4 (M)	—	—	—	—	—	—	0.2	0.2
NH_4HCO_3 (M)	1.0	1.0	1.0	1.0	1.0	1.0	—	—
Gas component								
CO_2 (cm Hg)	11	21	31	11	11	11	14	14
H_2 (cm Hg)	—	—	—	5	—	—	21	14
O_2 (cm Hg)	—	—	—	—	5	10	—	—
N_2 (cm Hg)	—	—	—	—	—	—	7	7
pH	7.70	7.70	7.70	7.70	7.70	7.70	4.80	4.80
pH (final)	7.30	7.52	7.30	7.45	7.40	7.40	7.30	7.21
Total-AMP yield (%) [*]	0.016	0.038	0.029	0.013	0.020	0.010	0.019	0.21
5'-AMP	1.2	1.3	1.3	1.4	1.3	1.1	1.5	1.6
2'(3')-AMP								

^{*} Total yield values (% adenosine converted to AMPs) contain relative errors of $\sim \pm 10\%$.

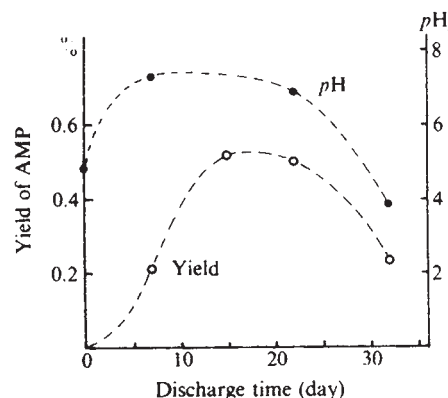


Fig. 3 Dependence of total yield of AMP, and of the pH of the solution, on the discharge time in run 8.

discharge time. The findings are shown in Fig. 3, together with measurements of pH. The initial increase of pH may be due to the formation of ammonia, and the decrease at the latter half to the formation of nitric acid (detected by the method using ferrous sulphate and concentrated sulphuric acid). The remaining solution was pumped out at the end of the run, and the vessel was washed with 10 ml of water. The pH of the washing was 0.05. The decrease of the yield at the end is probably due to the rather oxidative composition of the remaining gas, in which nitric acid and other oxidants would have been formed by the discharges. TLC and ion exchange chromatography showed many unknown substances, suggesting that adenosine decomposition took place.

As the results of these runs were dependent on the starting conditions, it seems possible that in certain conditions dehydration condensation could proceed linearly with discharge time. Studies to find such conditions are under way.

Received 9 July; accepted 21 September 1979.

1. Miller, S. L. & Orgel, L. E. *The Origin of Life on the Earth* (Prentice-Hall, Englewood Cliffs, 1974).
2. Schimpl, A., Lemmon, R. M. & Calvin, M. *Science* **147**, 149 (1965).
3. Schoffstall, A. M. *Origins of Life* **7**, 399 (1976).
4. Yamagata, Y. & Yamakawa, A. *Origin of Life, Proc. 2nd ISSOL Meet. and 5th ICOL Meet.* (ed. Noda, H.) p. 221 (Japan Sci. Soc. Press, 1978).
5. Lohrmann, R. & Orgel, L. E. *Science* **161**, 64 (1968).
6. Chen, P. S., Toribara, T. Y. & Warner, H. *Analyt. Chem.* **28**, 1756 (1956).
7. Heppel, L. A. *Meth. Enzym.* **2**, 570 (1955).

Nascent morphology of polyacetylene

Frank E. Karasz, James C. W. Chien,
R. Galkiewicz & Gary E. Wnek

Department of Polymer Science and Engineering,
Materials Research Laboratory, University of Massachusetts,
Amherst, Massachusetts 01003

Alan J. Heeger & Alan G. MacDiarmid

Laboratory for Research on the Structure of Matter,
University of Pennsylvania, Philadelphia, Pennsylvania 19104

Acetylene was first polymerised to a linear polymer in 1958¹, but the resulting grey powdery material, insoluble, infusible, and devoid of any interesting electrical properties, remained a scientific curiosity until Ito *et al.*² showed that it was possible to polymerise acetylene into a continuous film on the wall of a reactor. This polyacetylene film, (CH)_x, a large band gap semiconductor, can be transformed into a highly conducting material with $\sigma_{RT} \sim 1,000 (\Omega \text{ cm})^{-1}$ (refs 3–12) upon doping with either electron donors or acceptors^{13,14}. Although fibrous electron micrographs (EM) of unaligned² and partially aligned (stretch elongated) samples having enhanced conductivities have been reported³, it has been suggested that the observed morphology might be misleading, possibly resulting from a post-polymerisation handling of the film, or even an artefact such as the result of ultrasonication. The aim of this work is to determine the true nascent morphology of polyacetylene. We have polymerised acetylene directly on electron microscope gold grids and examined the polymer with scanning and transmission modes. The ultrathin specimen is entirely fibrous with diameters of ~5–40 nm, with 20-nm fibrils the most abundant. Some films show surface fractures revealing partially or completely aligned fibrils bridging the gaps. Some globular features were also observed suggesting local fusion. Thick films of polyacetylene also have fibrous morphology throughout. The observed structure is consistent with surface area measurements. Therefore, acetylene polymerises directly into fibrils, possibly due to the rigidity of the polymer backbone which is comprised of conjugated double bonds.

Acetylene was polymerised directly onto EM grids (3-mm disks of 300-mesh gold screen). This was accomplished by indium soldering of the grid on to a fine gold wire and hanging it in the reactor above the catalyst solution. The catalyst solution [7 mM Ti(OBu)₄, 28 mM Al(C₂H₅)₃] was introduced onto the grid as a thin liquid film by shaking. Acetylene gas (total pressure ranging from 10 to 760 torr of acetylene) was admitted for less than 1 min and pumped off immediately. The thickness of the polymer film may be controlled by varying the catalyst concentration, acetylene pressure and the time and temperature of the reaction. The ultrathin (CH)_x specimen thus prepared was washed to remove catalyst residues by repeated pentane distillation. A JEOL 100 CX Temscan electron microscope was used in this work. The four modes of observation were transmission (TEM), electron diffraction (ED), scanning (SEM) and scanning transmission (STEM). The polymerised film was observed by the first two modes; gold-coated films were examined with the other two modes. Gold coating was applied either by sputtering or evaporation with its thickness carefully controlled and kept in the 3–7-nm range.

Figure 1a is a STEM image at low magnification (10⁴) showing fibrous webs throughout the specimen. At higher magnification of 5×10^4 , Fig. 1b, c shows STEM images of the identical portion of the sample in which voids appear black (and white), respectively, demonstrating that the film is only a few fibrils in thickness. At 10⁵ magnification (Fig. 1d) individual fibrils were clearly discernible having diameters ranging from ~5 to 40 nm with 20-nm diameter fibrils being the most abundant. BET measurements (nitrogen gas) gave a surface area of 60 m² g⁻¹ which corresponds well with the observed fibre morphology.

Interestingly, polymerisations at higher acetylene pressure (760 torr) yield extremely smooth film on the grid even when observed at the highest magnifications. These films, however,

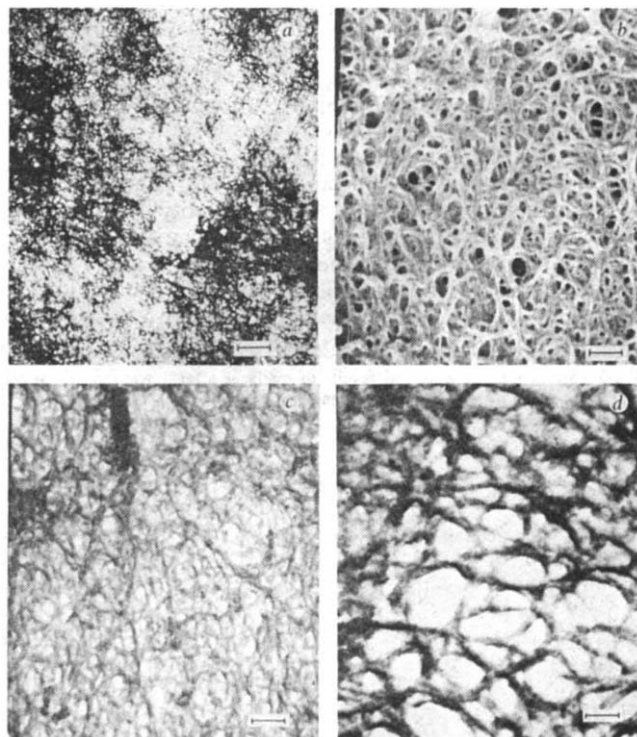


Fig. 1 a, Electron micrographs of ultrathin (CH)_x films polymerised directly onto a gold grid at 55 torr of acetylene for 30 s, dried and coated with a 3-nm layer of palladium gold by evaporation, $\times 10,000$ in the STEM mode. b, Same sample as in a at $\times 50,000$ SEM for matched areas. c, As for b but in STEM mode. d, Same sample as in a showing 100,000 STEM. Scale bars: a, 1 μm ; b, c, 0.2 μm ; d, 0.1 μm .

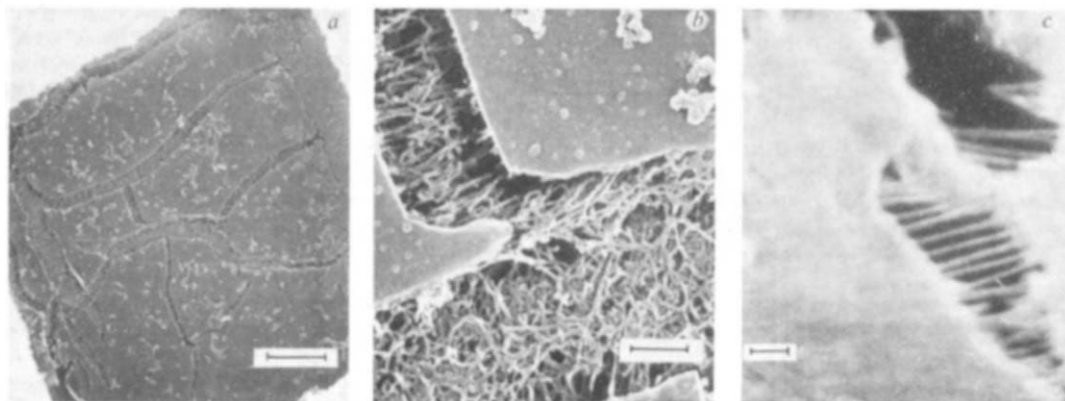


Fig. 2 SEM micrographs of thin $(\text{CH})_x$ films polymerised directly onto a gold grid at 760 torr of acetylene for ~ 1 min, dried and sputter coated with 3 nm of gold. *a*, $\times 1,500$; *b*, $\times 15,000$; *c*, $\times 100,000$. Scale bars: *a*, 10 μm ; *b*, 1 μm ; *c*, 0.1 μm .

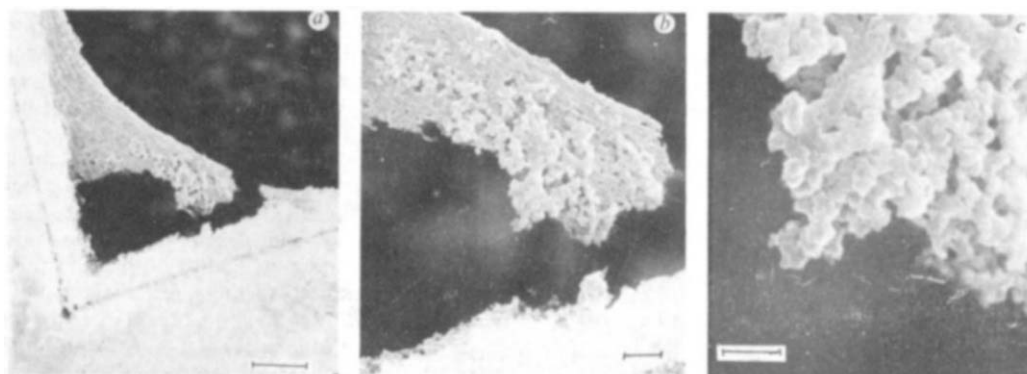


Fig. 3 SEM micrographs of another thin $(\text{CH})_x$ film polymerised as in Fig. 2. *a*, $\times 3,000$; *b*, $\times 8,000$; *c*, $\times 30,000$. Scale bars: *a*, 5 μm ; *b*, 1.25 μm ; *c*, 0.5 μm .

possess fractures (Fig. 2*a*) in which are found partially aligned fibrils (Fig. 2*b*). Occasional regions contain highly aligned 10-nm diameter fibrils (Fig. 2*c*). These fractures are probably formed during the washing procedure and are due either to the displacement of one diluent (toluene) with another (pentane) or to the thermal contraction occurring when the film was cycled between 78 K and room temperature. One explanation for the smooth film surface is that it consists of layers of $(\text{CH})_x$ polymerised in a substantially aligned state.

There are also globular structures, as shown, with increasing magnification, in Fig. 3. Examination of Fig. 3*a* shows that the globular growth appears to have been separated from a portion of the film as a result of melting contraction. Such local heating is entirely possible as the polymerisation is highly exothermic.

That the above morphology extends to thick continuous films formed by depositing a thin layer of catalyst solution on the wall of a cylindrical reaction vessel and subsequently exposing this layer to an atmosphere of acetylene, with polymerisation extending for several minutes, may be readily demonstrated. Such film has a highly reflecting metal-like surface on the side adjacent to the glass wall of the reactor (Fig. 4*a*) and a duller surface on the opposite side (Fig. 4*b*). The latter has the typical random fibrous morphology discussed above. The shiny side apparently has an identical morphology except that the fibrils are flattened and merged or fused into larger entities. Fusion is a distinct possibility even though one might expect heat transfer to be more efficient at the reactor wall. However, polymerisation is likely to occur very rapidly there because of a high catalyst concentration and lower monomer diffusion limitation.

Continuous $(\text{CH})_x$ film has a gross density of only $\sim 0.4 \text{ g cm}^{-3}$ whereas its flotation density is 1.16–1.18. Therefore, the film is highly porous with voids constituting two-thirds of the total

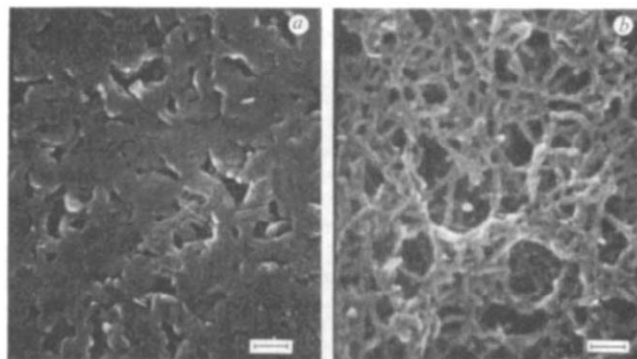


Fig. 4 SEM micrographs at $\times 50,000$ of thick $(\text{CH})_x$ film polymerised on the glass wall of reactor with 760 torr acetylene. *a*, Highly reflecting side of the film facing the wall; *b*, dull side of the film facing away from the wall. Scale bars, 0.2 μm .

volume. When the fibrils are not aligned, the gross morphology of $(\text{CH})_x$ film is somewhat analogous to the cellulose fibrils constituting a sheet of paper.

Ethylene and propylene polymerised by Ziegler–Natta catalysts have fibrillar, spherical, globular, worm-like, needle-shaped, and helical nascent morphologies depending on the type of catalyst and experimental conditions. Polyethylene fibres of 20–30 nM were obtained with $\text{TiCl}_3/\text{AlEt}_3$ catalyst¹⁴. Polypropylene fibrils of 30–50 nM diameter were formed by the

same catalyst¹⁵. The mechanism suggested was that the polymer chains formed on adjacent active sites of the α -TiCl₃ surface were insolubilised together into microfibrils¹⁶. The present Ti(OBu)₄/AlEt₃ catalyst has been described as an homogeneous or an heterogeneous system depending on experimental conditions¹⁷⁻¹⁹. The fact that uniform fibrillar polyacetylene was produced suggests an heterogeneous catalysis mechanism. Work is in progress to determine the dependence of nascent polyacetylene morphology on catalyst concentrations and polymerisation conditions.

We thank Professor E. L. Thomas for advice on electron microscopy.

Received 2 July; accepted 3 September 1979.

1. Natta, G., Mazzanti, G. & Corradini, P. *Atti. Acad. Nazl. Lincei Rend. Classe Sci. Fis. Mat. Nat.* **25**, 3 (1958).
2. Ito, T., Shirakawa, H. & Ikeda, S. *J. Polym. Sci. Polym. Chem. Ed.* **12**, 11 (1974).
3. *Chem. Eng. News*, p. 19, 24 April (1978).
4. Chiang, C. K. *et al. J. Am. chem. Soc.* **100**, 1013 (1978).
5. Chiang, C. D. *et al. Appl. Phys. Lett.* **33**, 18 (1978).
6. Chiang, C. K. *et al. J. chem. Phys.* **69**, 5098 (1978).
7. Park, Y. W. *et al. J. Polym. Sci. Polym. Lett. Ed.* **17**, 195 (1979).
8. Clarke, T. C., Geiss, R. H., Kwak, J. F. & Street, G. B. *J.C.S. chem. Commun.* 489 (1978).
9. Kwak, J. F., Clarke, T. C., Greene, R. L. & Street, G. B. *Bull. Am. phys. Soc.* **23**, 56 (1978).
10. Anderson, L. R., Pez, G. & Hsu, S. L. *J.C.S. chem. Commun.* 1066 (1978).
11. Hsu, S., Signorelli, A., Pez, G. & Baughman, R. J. *chem. Phys.* **69**, 106 (1978).
12. Park, Y. W., Denenstein, A., Chiang, C. K., Heeger, A. J. & MacDiarmid, A. G. *Solid State Commun.* **29**, 747 (1979).
13. Chiang, C. K. *et al. Phys. Rev. Lett.* **39**, 1098 (1977).
14. Ingram, P. & Schindler, A. *Makromolek. Chem.* **111**, 267 (1968).
15. Guttman, J. Y. & Guillet, J. E. *Polym. Preprints* **30**(1), 177 (1970).
16. Wristers, J. J. *Polym. Sci. Polym. Phys. Ed.* **11**, 1601 (1973).
17. Takeda, M., Imura, K., Nozawa, Y., Hisatome, M. & Koide, N. *J. Polym. Sci. C23*, 741 (1968).
18. Hirai, H., Hiraki, K., Noguchi, I. & Makishima, S. *J. Polym. Sci. A1* **8**, 147 (1972).
19. Hiraki, H., Kaneko, S. & Hirai, H. *J. Polym. Sci. Polym. Lett. Ed.* **10**, 199 (1972).

⁸⁷Rb-⁸⁷Sr chronology of the Binda howardite

J. L. Birck & C. J. Allegre

Laboratoire de Géochimie et Cosmochimie, Universités de Paris VI et VII, 4 Place Jussieu, 75230 Paris Cedex 05, France

Differentiated meteorites have numerous similarities with igneous rocks from the Earth and the Moon. They are of primordial importance in our understanding of magmatic processes in the early Solar System. The howardites constitute a particular class of differentiated meteorite as they are mechanical mixtures¹⁻⁷ (polymict breccia) of components from two other classes—eucrites and diogenites. The eucrites are representative of magmatic liquids and the diogenites are the corresponding cumulates³. This relationship is supported by oxygen isotopic data⁸. The ⁸⁷Rb-⁸⁷Sr chronology of eucrites^{9,10} shows that these objects result from a very early igneous activity near the surface of their parent body (<150 Myr after formation of the Solar System). As for howardites, within the same meteorite Kapoeta, basaltic clasts have been dated by the internal Rb-Sr isochron at 4,400 Myr and around 3,500 Myr (refs 11, 12). ³⁹Ar-⁴⁰Ar dating of these clasts provides evidence for their initial crystallisation 4,500 Myr ago, and for their independent history until the final lithification of Kapoeta, probably as late as 2,200 Myr ago¹³. Due to this complexity their initial age of crystallisation, and in particular, their ⁸⁷Sr/⁸⁶Sr initial ratio is not precisely known. We report here our investigation of the Binda howardite which seems to have had a simpler history; it is the only one which is a monomict breccia. The component representing the cumulate fraction seems to be in equilibrium with that which represents the eucritic melt³. Therefore one might expect to date the howardites formation by dating this meteorite, assuming that they all formed in the same parent body.

Minerals were separated from the meteorites by hand-picking and heavy liquid methods. Our experimental technique is similar to that used for the Lunar 24 basalt analysis¹⁴. Blanks are 2 ng, 0.004 ng, 0.03 ng for K, Rb and Sr, respectively. (Blank correction was never more than a few per cent.)

The results are given in Table 1, and Fig. 1 shows the conventional Rb-Sr diagram. Although the total rock samples of Binda are between those of Juvinas and Bereba¹⁰, concentrations in K, Rb and Sr are a factor of 2 to 5 lower than in these meteorites. The cumulate fractions are very poor in these elements⁷ and from our K-Rb-Sr concentration data, they must represent at least 50% of the Binda howardite. By plotting the data in the ⁸⁷Rb/⁸⁶Sr, ⁸⁷Sr/⁸⁶Sr diagram (Fig. 1) it may be seen that this object has undergone perturbation. However, all the points except one (PXH3) fall on a straight line within experimental error limits. This line corresponds to an age of $4,450 \pm 340$ Myr (2σ) with $\lambda = 1.42 \cdot 10^{-11} \text{ yr}^{-1}$ as decay constant for ⁸⁷Rb.

Heavy liquid can have preferential leaching effects on meteoritic and lunar minerals¹⁵. This can be understood if we assume that some fine particles become partially dissolved during the separation, because they are not separated from heavy liquids even when these are centrifuged. For achondrites^{9,10} and some lunar samples¹⁴ this seems to be the case for the light interstitial K-Rb rich phases, and might significantly affect the age. In the case of Binda, however, we believe that such an effect cannot produce our results. First, we have made a very rapid separation using small quantities of heavy liquids for Binda expecting that the effects increase with time. Second, short-time leaching by heavy liquids generally increases the model age of the leached fractions. Finally, both hand-picked and heavy liquid separates plot on the same straight line. We have also analysed the K, Rb and Sr contents of the liquors after the separations and (unlike ref. 15) they represent <1.3% of the total concentrations in the samples (Table 1). We conclude that the $4,450 \pm 340$ Myr age of Binda is not an experimental artefact and at least sets an upper limit for the age of the last remobilisation of the strontium but most probably dates this one.

The ⁸⁷Sr/⁸⁶Sr initial ratio calculated from whole rock assuming an age of 4,470 Myr is 0.69894 ± 0.00004 . Similarly, if the hand-picked plagioclase 'Plag H' is assumed to have the same age, this indicates an ⁸⁷Sr/⁸⁶Sr initial ratio of 0.69894 ± 0.00004 . This value is indistinguishable from that for eucrites¹⁰⁻¹⁶. The small insignificant difference might be due to a non-representative whole rock sample. A small excess of pyroxene in the latter may explain the difference. One can compare this ⁸⁷Sr/⁸⁶Sr initial ratio to that for the other howardite studied so far: Kapoeta^{11,12}. That for Kapoeta is significantly lower in clast C which has an age of 4,440 Myr and ⁸⁷Sr/⁸⁶Sr initial ratio of 0.69885 ± 0.00005 . The Sr initial ratio for clast A is almost as low as that for clast C. We are faced with two alternatives. First that this low initial value is real. In this case the basaltic clasts (A and C) are not related to the magmatism which created Binda and the eucrites. They may come from another part of the same parent body or Kapoeta comes from another parent body with a similar history with a different timing. In any case, Kapoeta contains exotic components like chondritic material¹, and its formation seems to involve many components¹⁷ of various origins. The alternative interpretation is that this low initial ratio is insignificant and results from Kapoeta being a mixture of different components of various ages; a similar interpretation has been discussed for lunar samples¹⁸. We cannot distinguish between these possibilities at present.

As in eucrites, the ⁸⁷Rb-⁸⁷Sr mineral age for Binda is controlled by a light K-rich phase. The internal age might simply relate to this mineral. One can interpret this younger age as a secondary resetting of the ⁸⁷Rb-⁸⁷Sr clock by metamorphism or shock, 3,450 Myr ago. This result is similar to the ⁸⁷Rb-⁸⁷Sr and ³⁹Ar-⁴⁰Ar ages around 3,800 and 3,500 Myr of some clasts of Kapoeta¹¹⁻¹³. It also agrees with the 3,600 Myr ³⁹Ar-⁴⁰Ar age of Malvern¹⁹. At the opposite, the ³⁹Ar-⁴⁰Ar ages of the

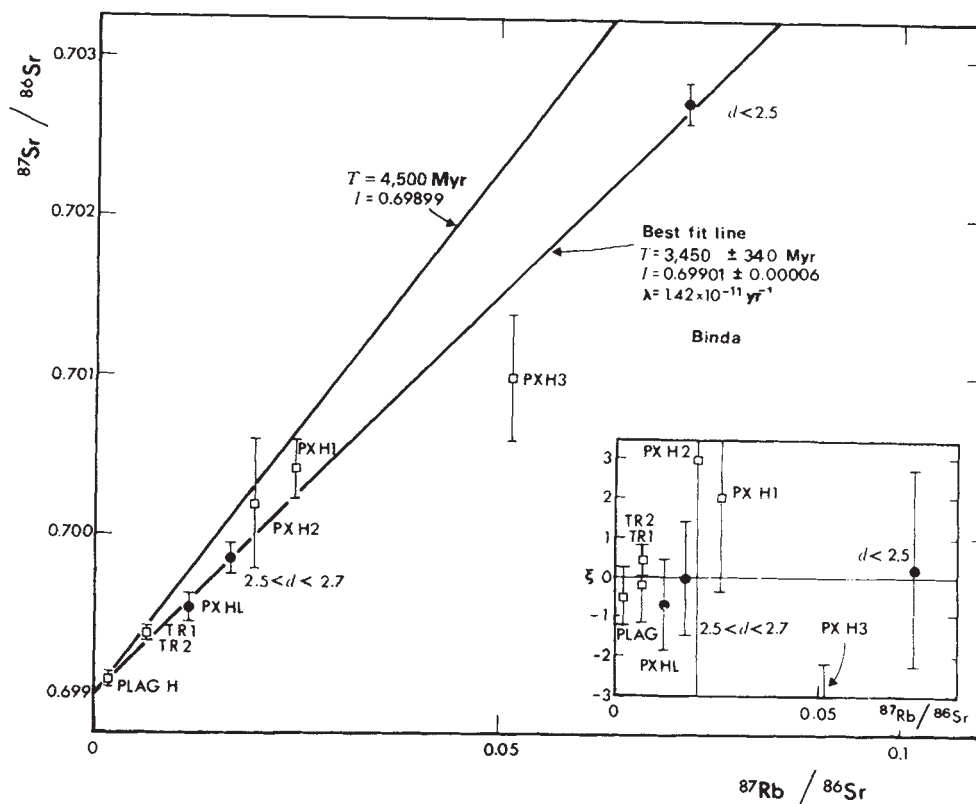


Fig. 1 Rb-Sr evolution diagram for the minerals of the Binda howardite. The decay constant used here for ^{87}Rb is $\lambda = 1.42 \times 10^{-11} \text{ yr}^{-1}$. TR, total rock; PX, pyroxene; Plag, plagioclase. The inset shows the relative deviation in 10^{-4} units of the experimental points from the best fit line as a function of $^{87}\text{Rb}/^{86}\text{Sr}$. The reduced χ^2 , σ is 0.84 and shows that the points can be fitted to a straight line within experimental errors. The anticorrelation between the errors of slope and intercept is $s = -0.639$ (see ref. 23). ●, Heavy liquids; □, hand-picked.

Table 1 Experimental results for Binda

Sample	Weight analysed	K (p.p.m.)	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}^*$	$^{87}\text{Sr}/^{86}\text{Sr}^\dagger$
Total rock 1	40.0	80.0	0.0925	39.7	0.00643	0.69936 (3)
Total rock 2	23.3	68.7	0.0752	33.63	0.00647	0.69932 (7)
Pyroxene H1‡	10.2	2.94	0.00593	0.690	0.0249	0.70040 (17)
Plagioclase H‡	2.0	267.0	0.0897	135.2	0.00192	0.69907 (5)
Pyroxene H2‡	21.0	3.29	0.00657	0.977	0.0194	0.70019 (44)
Pyroxene HL‡	45.1	13.1	0.0243	6.08	0.01159	0.69954 (8)
Pyroxene H3‡	6.0	6.47	0.0169	0.951	0.0517	0.70099 (40)
$2.5 < d < 2.7$ §		166.0	0.212	36.6	0.0168	0.69985 (10)
$d < 2.5$ §		95.0	0.317	12.50	0.0734	0.70271 (18)
Heavy liquid content§		285.0	0.40	2.5	0.46	

* The error on this ratio is 1%.

† Errors are 2σ quoted on the last decimal place.

‡ H, handpicked; HL heavy liquid separates.

§ These data are given in nanograms because the weight is unknown.

|| The heavy liquid (methylene iodide + acetone) was in contact with 320 mg of powder.

howardites Bununu and Yamato L are higher than 4,200 Myr (ref. 19) and 4,000 Myr (ref. 20) respectively. Similarly ^{39}Ar - ^{40}Ar dating of Kapoeta also yields ages $\geq 4,500$ Myr (ref. 13). If all howardites are genetically related, these data show that they probably crystallised 4,500 Myr ago and were later disturbed by one or several large scale secondary events 3,400–3,800 Myr ago which are probably related to impacts. As discussed by Wetherill²², the absence of a cluster of resetting ages around 4,000 Myr is not a conclusive argument against an inner Solar System wide 'cataclysm' such as determined on the Moon²¹. However, there is a lot of evidence for large-scale impacts in Basaltic Achondrites between 3,900 and 3,500 Myr which are not so far recorded in the lunar rocks actually analysed.

We thank S. Clark for the meteorite sample and J. F. Minster and N. Shimizu for helpful discussions.

Received 9 May; accepted 18 September 1979.

- Shimizu, N. & Allegre, C. J. *Meteoritics* **10**, 488 (1975).
- Dreibus, G., Kruse, H., Spettel, B. & Wanke, H. *Geochim. cosmochim. Acta Suppl.* **8**, 211–227 (1977).
- Stolper, E. *Geochim. cosmochim. Acta* **41**, 1271–1282 (1977).
- Moore, C. B. in *Meteorites* (Wiley, New York, 1962).
- MacCarthy, T. S., Ahrens, L. H. & Erlank, A. J. *Earth planet. Sci. Lett.* **15**, 86–93 (1972).
- Taylor, H. P., Duke, M. B., Silver, L. T. & Epstein, S. *Geochim. cosmochim. Acta* **29**, 489–512 (1965).
- Allegre, C. J., Birck, J. L., Fourcade, S. & Semet, M. *Science* **187**, 436 (1975).
- Birck, J. L. & Allegre, C. J. *Earth planet. Sci. Lett.* **39**, 37–51 (1978).
- Papanastassiou, D. A., Rajan, R. S., Huneke, J. C. & Wasserburg, G. J. in *Lunar Science V*, 2, 583 (Lunar Science Institute, Houston, 1974).
- Papanastassiou, D. A. & Wasserburg, G. J. in *Lunar Science VII*, 2, 665 (Lunar Institute, Houston, 1976).
- Rajan, R. J., Huneke, J. C., Smith, S. P. & Wasserburg, G. J. *Geochim. cosmochim. Acta* **43**, 957–971 (1979).
- Birck, J. L. & Allegre, C. J. *Phys. Earth planet. Int.* **16**, 10–14 (1978).
- Papanastassiou, D. A. & Wasserburg, G. J. *Geochim. cosmochim. Acta Suppl.* **6**, 1467–1489 (1975).
- Papanastassiou, D. A. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **5**, 361–376 (1969).
- Dymek, R. F., Albee, A. L., Chodos, A. A. & Wasserburg, G. J. *Geochim. cosmochim. Acta* **40**, 1115–1130 (1976).
- Birck, J. L., Fourcade, S. & Allegre, C. J. *Earth planet. Sci. Lett.* **26**, 29–35 (1975).
- Rajan, R. S., Huneke, J. C., Smith, S. P. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **27**, 181–190 (1975).
- Balacescu, A. & Wanke, H. *Meteoritics* **12**, 171–172 (1977).
- Tera, F., Papanastassiou, D. A. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **22**, 1–21 (1974).
- Wetherill, G. W. *Geochim. cosmochim. Acta Suppl.* **6**, 1539–1561 (1975).
- Minster, J. F., Ricard, L. P. & Allegre, C. J. *Earth planet. Sci. Lett.* (in the press).

- Duke, M. B. & Silver, L. T. *Geochim. cosmochim. Acta* **31**, 1637–1665 (1967).
- Allegre, C. J., Shimizu, N. & Treuil, M. *Phil. Trans. R. Soc. A* **285**, 55–67 (1977).

Allende meteorite—old age but normal isotopic composition of potassium

W. Stegmann & F. Begemann

Max-Planck-Institut für Chemie (Otto-Hahn-Institut), Mainz, FRG

The extensive investigation of the Allende meteorite has provided much new information on the early history of our Solar System. The findings include small but significant anomalies of the isotopic composition for several elements which are clearly not due to any of the processes hitherto known to cause such variations (see ref. 1 for review). These anomalies seem to confirm the reality of astrophysical concepts developed since the classical paper of Burbidge *et al.*² on the different modes of origin of the nuclei. Evidence has also been found which points to the possible presence in Allende of pre-solar condensates. Jessberger and Dominik³ have presented data to support an earlier result⁴ according to which the ⁴⁰Ar gas retention ages of some white inclusions from Allende exceed the canonical age of the Solar System. These gas retention ages were obtained by ⁴⁰Ar–³⁹Ar dating, however, and are therefore based on the assumption that the ³⁹K/⁴⁰K ratio in the inclusions is identical to that used in the reference standard. In view of the far-reaching implications of finding in meteorites solid matter pre-dating by roughly 500 Myr the condensation of the Solar System we have attempted to verify this assumption. This is especially important because the Allende inclusions contain elements with an anomalous isotopic composition.

We were given (by E. K. Jessberger) two portions of inclusion 18: three chips with a combined weight of 1.9 mg and another aggregate of 16 mg. Although these portions are not true aliquots of a well homogenised sample they are, nevertheless, considered representative of the sample analysed by Jessberger and Dominik.

The small sample was used for pilot experiments. One chip (780 µg) was treated in a small Teflon bomb with a mixture of 5 µl HF (40%) and 3 µl HClO₄ (70%) for 5 h at 190 °C. It was fumed to dryness in the Teflon bomb and taken up with 20 µl HF (10%). Part of the solution was spiked with both ⁴⁰K and ⁴²Ca for the determination of the concentrations of potassium and calcium by isotope dilution, the remainder was used for the potassium analysis. If there had been a residue its distribution

among the spiked and the unspiked fraction may have been inhomogeneous.

Although using HF to take up the sample drastically reduced the Ca⁺/K⁺ ratio in the mass spectrometer, the interference on mass 40 from ⁴⁰Ca⁺ could not be entirely neglected. Hence, from all subsequent samples with one exception (see below) the potassium was separated by ion exchange. This resulted in very weak signals on the monitor masses 42 and 44 but, because of the high ⁴⁰Ca/⁴²Ca and ⁴⁰Ca/⁴⁴Ca ratios respectively, it caused a large uncertainty in the residual interference on mass 40. This was avoided by adding a ⁴²Ca spike to the potassium after it had been separated on the ion exchange column. (The resulting ⁴⁰Ca/⁴²Ca ratio itself was measured after termination of the potassium measurement by increasing the temperature of the sample filament until Ca dominated the mass spectrum.) The spike solution contained 10^{−3} g K of normal isotopic composition per gramme of ⁴²Ca.

The remaining two chips from the small sample (1,100 µg) were treated as before (10 µl HF + 4 µl HClO₄, 5 h at 180 °C, fuming to dryness, 5 µl each of HF and HClO₄, 10 h at 190 °C, fuming to dryness). The residue was taken up with 20 µl of 0.5 M HCl. Part of it was spiked with ⁴⁰K and ⁴²Ca, the rest (95%) was filtered through a Teflon filter (Millipore FH 0.5 µm) and the filtrate put on a quartz ion-exchange column (3 mm diameter, length 5 cm) filled with Dowex 50 WX 8. Potassium was eluted with 0.9 M HClO₄. The eluate (0.63 ml) was spiked with 12 ng of ⁴²Ca, it was evaporated to dryness and dissolved again in 5 µl HF (10%) for the mass spectrometric measurement.

The 16 mg aggregate was decomposed as described above except that larger amounts of acid were used and that the heat treatments in the Teflon bomb were 48 h and 15 h, respectively. After the last fuming to dryness the residue was taken up in 530 µl of 0.9 M HClO₄ and centrifuged. About 1% of the supernatant was spiked with ⁴⁰K and ⁴²Ca, the bulk put first through a large (4 mm diameter, length 5 cm) and then a small ion exchange column. In both instances potassium was again eluted with 0.9 M HClO₄ and ⁴²Ca was added as before. The undissolved portion of the aggregate, the residue after centrifuging, amounted to <1% of the original 16 mg. It was brought into solution by treating it in the Teflon bomb for 16 h at 190 °C with 5 µl each of H₂SO₄ (96%) and H₃PO₄ (85%). It was analysed by mass spectrometry without any more chemistry.

Only quartz and Teflon were used throughout the experiments; all acids were Merck grade 'suprapur'. Their K-content was determined by isotopic dilution and was found to be for HF (40%) 4 p.p.b. (parts per 10⁹); HClO₄ (70%) 50 p.p.b.; HCl (4.5 M) 1 p.p.b.; H₂SO₄ (96%) 80 p.p.b.; H₃PO₄ (85%) 140 p.p.b. and H₂O 1 p.p.b. The total blank of dummy runs with

Table 1 Elemental concentrations of K and Ca and isotopic composition of potassium

Sample	(mg)	Run	Detector*	K (p.p.m.)	Ca (%)	³⁹ K/ ⁴¹ K		⁴¹ K/ ⁴⁰ K _{corr}	I(⁴⁰ Ca)/I(40)	K-blank (%)
						Max	Min			
Terrestrial		112	M	—	—	14.099–13.899	573.7 ± 7.1	—	—	—
		112	F	—	—	13.800–13.727	586.0 ± 10.0	—	—	—
JD18-SI	0.78	114	M	85 ± 20	11.3 ± 1.2	14.405–13.819	562.4 ± 13.6	—	≤ 8 × 10 ^{−2}	≤ 3
Terrestrial		116	F	—	—	13.862–13.541	585.1 ± 3.6	—	—	—
Terrestrial		131	M	—	—	14.329–14.167	572.7 ± 10.8	—	—	—
		131	F	—	—	13.935–13.703	585.4 ± 6.2	—	—	—
Terrestrial		134	M	—	—	14.138–14.090	576.9 ± 4.1	—	—	—
JD18-SII	1.10	135	M	21 ± 4	8.3 ± 0.8	14.311–14.066	574.0 ± 5.5	—	≤ 1.2 × 10 ^{−4}	≤ 43
Terrestrial		141	M	—	—	14.304–14.220	575.3 ± 2.1	—	—	—
JD18-SIII	15.93	142	M	114 ± 20	13.0 ± 1.2	14.194–14.100	572.1 ± 3.0	—	≤ 2.4 × 10 ^{−4}	≤ 0.6
Terrestrial		144	F	—	—	13.860–13.807	581.6 ± 4.6	—	—	—
Terrestrial		146	M	—	—	14.306–14.145	568.4 ± 14.4	—	—	—
JD18-SIII	15.93	147	M	114 ± 20	13.0 ± 1.2	14.219–14.160	573.0 ± 6.7	—	≤ 0.6 × 10 ^{−4}	≤ 0.6
		147	F	—	—	13.832–13.712	582.3 ± 2.2	—	—	—
Terrestrial		148	F	—	—	13.902–13.850	585.5 ± 1.5	—	—	—
JD18-SIIIR		150	M	—	—	14.411–14.138	579.5 ± 2.4	—	≤ 2 × 10 ^{−3}	—

The ⁴¹K/⁴⁰K ratios are corrected for mass discrimination only, their errors are ± 1 σ (mean standard deviation). Sample SIIIR is the residue from SIII.

* M, secondary electron multiplier, F, Faraday cup.

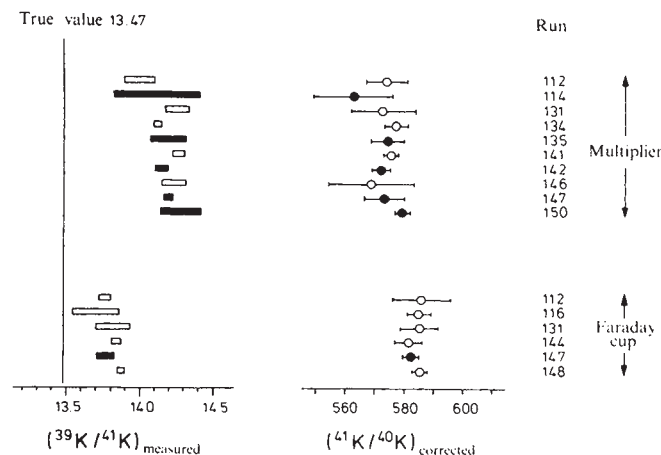


Fig. 1 Measured $^{39}\text{K}/^{41}\text{K}$ ratios and mass discrimination-corrected $^{41}\text{K}/^{40}\text{K}$ ratios of terrestrial (open symbols) and Allende (filled symbols) samples. The length of the bars for the measured $^{39}\text{K}/^{41}\text{K}$ ratios do not indicate the errors of these ratios but rather the shift with time observed during the measurement. The Faraday cup measurements are in complete agreement with the results of Burnett *et al.*¹⁰ and Begemann and Stegmann⁷ who, after normalisation to the same $^{39}\text{K}/^{41}\text{K}$ ratio of 13.47, reported values of 584.89 and 584.87, respectively.

appropriate amounts of acids was ≤ 2 ng K, passing the solution through the ion-exchange columns increased the value to ≤ 10 ng.

The mass spectrometric measurements were performed by surface ionisation using double (rhenium) filament sources. Only in the case of the 16 mg sample was the ion current high enough for a Faraday cup, together with a $10^{11}\Omega$ resistor, to be used as a detector. This necessitated a correction for the nonlinear voltage dependence of the resistor; a second order polynomial gave a fit through the experimental data to better than 3×10^{-4} for the whole range of interest (10–65 V). In all other instances a 16-stage secondary electron multiplier (1,500–1,800 V, $10^9\Omega$, gain $\approx 2 \times 10^5$) was used where no such correction was necessary. Data acquisition was by computer-controlled peak jumping using a 5 $\frac{1}{2}$ -digit integrating voltmeter. Details will be published elsewhere⁵.

The results are compiled in Table 1. The potassium contents in column 4 are uncorrected for blanks, the last column gives upper limits for the contribution of blank potassium to the total, based on 2 ng K for samples SI and SIIIR and 10 ng for SII and SIII (see above). Owing to the low ion currents in the mass spectrometer a considerable fraction of the total potassium had to be measured. Consequently, a marked shift with time of the isotope ratios was observed. This is indicated by the range of $^{39}\text{K}/^{41}\text{K}$ ratios in column 7, where the highest values are the initial ones. The $^{41}\text{K}/^{40}\text{K}$ ratios are corrected for fractionation by adopting as true value $^{39}\text{K}/^{41}\text{K} = 13.47$ (ref. 6) and assuming the mass discrimination between neighbouring isotopes to be constant. In no case was there any positive evidence for a contribution to mass 40 from $^{40}\text{Ca}^+$; the limits which can be set are indicated in column 9.

Figure 1 shows the isotope data of Table 1, together with results for normal terrestrial K as obtained on samples of similar size as the meteoritic ones. The degree of the instrumental fractionation can be seen from the off-set of the measured $^{39}\text{K}/^{41}\text{K}$ ratios from the Nier value of 13.47. Particularly obvious is the additional discrimination caused by the mass dependent gain for different isotopic species of the multiplier. It also seems that the corrected $^{41}\text{K}/^{40}\text{K}$ ratios are systematically lower for the multiplier compared with the ones with the Faraday cup as collector. Contributions on mass 40 from species other than ^{40}K can be excluded as was verified by large terrestrial samples. The reason for this discrepancy is not clear. As

both the terrestrial and the meteoritic samples are affected, however, there is no potential reason apparent for any misinterpretation as long as both sets of data are considered separately.

We conclude from these data that within the experimental limits of error the isotopic composition of potassium from the Allende inclusion is indistinguishable from that of normal potassium. Neither the $^{39}\text{K}/^{41}\text{K}$ nor the $^{41}\text{K}/^{40}\text{K}$ ratio shows any evidence for an anomaly in excess of 1%. As pointed out by Jessberger and Dominik³, for a sample 4,600 Myr old to yield an apparent ^{40}Ar – ^{39}Ar age of 5,100 Myr the ^{40}K would have to be enriched by about 35%. As our upper limit to a possible anomaly falls short of this by more than an order of magnitude the old apparent age of this inclusion cannot be due to an anomalous abundance of ^{40}K .

As one of the assumptions underlying the ^{40}Ar – ^{39}Ar method has been verified the old apparent ages of some of the Allende inclusions are more likely to be the true ones. Unfortunately, however, the interpretation is still ambiguous as another prerequisite for obtaining true ages is that all ^{40}Ar from the sample is indeed produced by the *in situ* decay of ^{40}K . We feel that it would be premature to exclude the possibility of the presence in meteoritic matter of extraneous 'unsupported' or 'orphan' ^{40}Ar .

We should re-emphasise that in the inclusion investigated the relative abundance of ^{41}K agrees with the normal value to within $\pm 1\%$. Thus there is no evidence^{7,8} to support Clayton's suggestion⁹ that pre-solar grains might contain excess ^{41}K from the *in situ* decay of ^{41}Ca . This result will be particularly important if the ^{40}Ar – ^{39}Ar ages should turn out to be the true ages as this would make the inclusions, or part of them, true presolar condensates.

Received 1 August; accepted 21 September 1979.

1. Clayton, R. N. A. *Rev. nucl. Part. Sci.* **28**, 501–522 (1978).
2. Burbidge, E. M., Burbidge, G. R., Fowler, W. A. & Hoyle, F. *Rev. mod. Phys.* **29**, 547–650 (1957).
3. Jessberger, E. K. & Dominik, B. *Nature* **277**, 554–556 (1979).
4. Jessberger, E. K., Staudacher, Th., Dominik, B. & Herzog, G. F. *Meteoritics* **12**, 266–269 (1977).
5. Stegmann, W. thesis, Univ. Mainz (1979).
6. Nier, A. O. *Phys. Rev.* **77**, 789–793 (1950).
7. Begemann, F. & Stegmann, W. *Nature* **259**, 549–550 (1976).
8. Birck, J. L., Lorin, J. C. & Allegre, C. *Meteoritics* **12**, 179–180 (1977).
9. Clayton, D. D. *Nature* **257**, 36–37 (1975).
10. Burnett, D. S., Lippolt, H. J. & Wasserburg, G. J. *J. geophys. Res.* **71**, 1249–1269 (1966).

Age and significance of alluvium in the Windrush valley, Oxfordshire

J. Hazelden & M. G. Jarvis

Soil Survey of England and Wales, Rothamsted Experimental Station, Harpenden, Hertfordshire, UK

The floodplain deposits of the upper Thames and its tributaries commonly comprise 0.5–1 m of clay alluvium overlying, with a sharp boundary, a variable thickness of sandy limestone gravel. Samples of wood (probably alder root) from the top of the gravel in a temporary exposure (SP 360084) in the floodplain of the river Windrush, south of Witney, Oxfordshire, gave a radiocarbon date of $2,660 \pm 85$ yr BP (I-9337); these roots had been truncated at the gravel/clay interface. Here we consider the sequence of events and the possible causes of this change to clay deposition; we conclude that the latter resulted chiefly from a rapid increase in local forest clearance and in the amount of ploughed land, after 2,660 yr BP.

The Windrush is a tributary of the Thames; it drains the southern edge of the Cotswolds, Jurassic limestone hills north and west of Witney, and then flows southeastwards across the Oxford Clay country to the Thames at Newbridge (SP 403015).

Its valley is characteristically narrow and entrenched within the Cotswolds but widens to about 1 km where it crosses the undulating clay land south of Witney.

The solid geology of the district is fairly simple. Thin Cornbrash limestone outcrops beneath Witney and to the north, with older limestones and clays of the Great Oolite series exposed locally in small folds and faults. These strata dip gently to the south-east and are overlain by Oxford Clay south of Witney. Drift deposits are extensive, especially the limestone gravels on river terraces.

About 1 m of Flandrian clay alluvium overlies the gravel in the Windrush floodplain adjacent to the river. Locally layers of peat, up to 0.3 m thick, occur at the junction of these two deposits, especially near the margins of the floodplain. Auger bores and occasional pits have proved this sequence of deposits throughout the Windrush floodplain from Witney to the Thames.

The site we sampled showed 1.06 m of clay alluvium over about 5 m of sandy limestone gravel, in turn probably over Oxford Clay (not seen); the gravel contained scattered organic matter. The root fragments were up to 0.04 m in diameter and seemed to be in position of growth in the gravel. They were truncated, only one fragment protruding some 0.1 m above the gravel surface.

These deposits suggest the following sequence of events. At first a high energy stream deposited the sandy gravel. Then followed peat accumulation, either locally in quiet backwaters or perhaps as a widespread deposit subsequently largely removed by erosion. Elsewhere in the Windrush floodplain we have seen large, unidentified logs in this peat, up to 0.1 m diameter. In either event the top of the gravel was eroded, at least locally, as in the section we studied the roots had been broken off and no tree stumps remained. The last event in this sequence was deposition of clay alluvium. A dark layer within it, about 0.5 m above the gravel and 0.07 m thick, represents an old topsoil—a level at which deposition temporarily slowed or ceased allowing organic matter to accumulate at the ground surface.

The radiocarbon date is significant for the age of the clay alluvium. Clearly the alder fragments post-date the gravel in which they grew, but are older than the period of erosion and the subsequent deposition of clay. The beginning of accumulation of clay alluvium at this site was, therefore, after 2,660 yr BP.

Corroborative evidence for changing conditions near or after this date is available at Farmoor, on the Thames 8 km east of this site. Here the Oxfordshire Archaeological Unit excavated an Iron Age site, dated to around 200 BC, which lies above the gravel surface but is buried by clay alluvium 0.3–1 m thick; mollusc, beetle and seed remains suggested that the site became very wet around 200 BC or possibly earlier¹.

Similar radiocarbon dates to ours have also been obtained from alluvial deposits, of a slightly different nature, at Ipsley, Worcestershire^{2,8} at Pilgrim Lock, Warwickshire^{3,8} and at Worcester^{3,8}, although here the alluvium seems to have accumulated at a faster rate than in the Windrush floodplain.

Although the sequence of events on the Windrush floodplain is reasonably clear, the cause of the change from the period of erosion which truncated the alder roots to the period of deposition of the clay is not. Four possible causes are considered below: lateral movement of the stream within its valley, a rise in sea level relative to the land, a change in climate, or a response to human activities.

Floodplain conditions in the Witney district were fairly uniform; the same sequence of deposits is recorded throughout much of the valley, and thus the changes must have been in response to factors of more than local significance. The available evidence⁴ suggests that sea-level changes during this latter part of the Flandrian were very small, and unlikely to have significantly affected a river this far inland. The worsening of climate (higher rainfall) at about this time, the beginning of the sub-Atlantic period^{5–7} could have been a contributing factor⁵ but such changes alone are an unlikely cause. Climatic changes

were possibly responsible for the cessation of erosion but there is no supporting evidence for the major change which would have been necessary to bring about this change in the nature of deposition, which seems to have lasted to the present, as the alluvium is still accumulating.

Although the deposits here are somewhat different our conclusions are similar to those of Shotton⁸. We suggest that the deposition of clay alluvium here began chiefly as a result of man's activity, probably a rapid increase in land clearance and cultivation (ploughing). This would have increased soil erosion, and have meant more rapid surface run-off and increased likelihood of short-term flooding in the river valley. Run-off would have been greatest over clay (impermeable) ground, so that much of the detritus carried to the river valley would be clay-sized (<2 µm) particles. This increase in land clearance and cultivation and the consequent deposition of the clay alluvium began after 2,660 yr BP.

Received 27 July; accepted 1 October 1979.

1. Lambrick, G. & Robinson, M. *Iron Age and Roman Riverside Settlements at Farmoor, Oxfordshire*, 111, 118 and 141 (Oxfordshire Archaeological Unit Report No 2, 1979).
2. Shotton, F. W. & Williams, R. E. G. *Radiocarbon* **13**, 141–156 (1971).
3. Williams, R. E. G. & Johnson, A. S. *Radiocarbon* **18**, 249–267 (1976).
4. Shephard-Thorn, E. R. *Proc. geol. Ass.* **86**, 537–547 (1975).
5. Kerney, M. P., Brown, E. H. & Chandler, T. J. *Phil. Trans. B* **248**, 135–204 (1964).
6. Starkel, L. in *Proc. Int. Symp. on World Climate 800–0 BC* (ed. Sawyer, J. S.) 15–33 (London, 1967).
7. Evans, J. G. *The Environment of Early Man in the British Isles*, 145–7 (Paul Elek, London, 1975).
8. Shotton, F. W. in *The Effect of Man on the Landscape: the Lowland Zone* (eds Limbrey, S. & Evans, J. G.) 27–32 (Council for British Archaeology, Research Report No 21, 1978).

Oxygen 18 in estuaries

J. M. Martin

Laboratoire de Géologie, Ecole Normale Supérieure 46 rue d'Ulm, 75230 Paris Cedex 05, France

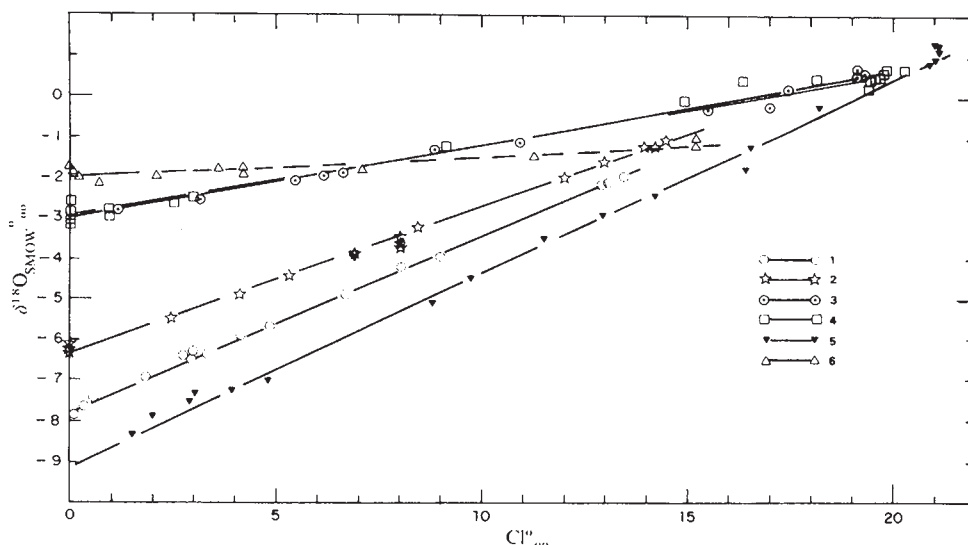
R. Letolle

Laboratoire de Géologie Dynamique, Université Pierre et Marie Curie, 4 place Jussieu, 75230 Paris Cedex 05, France

A basic approach used in estuarine chemistry is to determine the experimental distribution of one given element with chlorinity and compare it with that predicted from the simple mixing of river water with seawater. This method provides useful results but several practical problems arise—including the definition of correct end-members, the role of input of tributaries or bank flow in the saline part of the estuary, and the occurrence of several water masses of different ages along the estuary. In addition, this method is not applicable in those areas of constant density which can be of prime importance in estuarine geochemistry and dynamic understanding. To characterise these various problems it is worthwhile to use a suitable freshwater tracer such as tritium¹, D/H or the ¹⁸O/¹⁶O isotopic ratio. The oxygen isotope ratio has been used successfully to study river water mixing² but has been little used in estuaries. The basic principle of this method is that the isotopic composition of river water might vary with time, and that different rivers might have different isotopic composition whereas ocean water is of fairly uniform composition. We present here a study of oxygen isotope composition in various estuaries, ranging (in Pritchard's classification³) from the saltwedge type such as Rhône (France) and Zaire (Congo), to a partially mixed type such as Gironde, Loire and Charente (France) and Godavari (India). These estuaries cover a wide variety of hydroclimatological conditions including the temperate and tropical regimes.

Samples were collected in Niskin bottles, or with a plastic bucket for surface samples. Aliquots of 25 ml were stored in tightly closed glass bottles for ¹⁸O analysis and larger volumes were kept in polyethylene bottles for chlorinity measurements.

Fig. 1 ^{18}O content and chlorinity for: 1, Loire (30/04/1970); 2, Loire (23/11/1970); 3, Zaire (9–19/11/1976); 4, Zaire (7–13/05/1978); 5, Rhône (01–05/02/1978); 6, Godavari (13–17/12/1978).



The analytical procedure used in determining the $^{18}\text{O}/^{16}\text{O}$ isotopic ratio involves the equilibration of 3 ml of water with carbon dioxide gas at 25 °C and analysing a small aliquot of the gas in a mass spectrometer (VG 602 C)⁴. Oxygen isotopic ratios are expressed as per mil deviation ($\delta^{18}\text{O}$) from standard mean ocean water (SMOW), and the absolute accuracy of the method is ± 0.05 ‰.

Results of the analyses are presented in Figs 1–3 which systematically show the correlation of $\delta^{18}\text{O}$ with chlorinity. The estuarine classification based on the chlorinity structure¹ can be expanded by data on $\delta^{18}\text{O}$ distribution with chlorinity. It is possible to distinguish two categories of estuary:

(1) Estuaries with an isotopically homogeneous freshwater component (Fig. 1): in these estuaries the $\delta^{18}\text{O}$ experimental points fit fairly well the theoretical dilution curve. This most common type is observed for Zaire and Rhône estuaries which are typical salt-wedge estuaries according to chlorinity distribution. Results obtained in the Loire⁵ and Godavari estuaries are given for comparison. These estuaries which are partially mixed and occasionally stratified according to chlorinity structure show a strict correlation between ^{18}O and chloride content. Thus in these four estuaries there is a perfect mixing between river and ocean water molecules. The slope of the regression curve for the Godavari estuary is much smaller, and extrapolation to the ocean water end-member—which it was not possible to sample at that time—gives values much lower than those shown on the Atlantic Ocean. However, a chlorinity end-member of 18.3‰ (ref 6) corresponds to a $\delta^{18}\text{O}$ of -1.0 using the Craig and Gordon equation⁷ for the Pacific ($\delta^{18}\text{O} = 0.68S -$

23.5); the same using a similar equation developed by Duplessy⁸ on the South Indian Ocean ($\delta^{18}\text{O} = 0.66S - 22.6$) would give -1.5 ‰. Therefore the Godavari regression line appears completely compatible with the local ocean setting.

(2) Estuaries with an isotopically heterogeneous freshwater component (Figs 2, 3): in this type of estuary $\delta^{18}\text{O}$ value of estuarine waters corresponds to the mixing of ocean waters with river water of various ages or origins: this has been observed in the Charente (1973) and Gironde (March 1978) where the rate of tidal mixing cannot smooth the small variations in river water isotope composition (Fig. 2). Moreover, in the Gironde (October 1978) and Charente (1974) (Fig. 3) well differentiated water masses can be identified. Considering first the Gironde (October 1978) waters with a chlorinity higher than 1‰ result from the mixing of ocean water with river water of $\delta^{18}\text{O} = -7$ ‰; below this chlorinity, the original river water has a $\delta^{18}\text{O}$ of -7.5 ‰. In the Charente (1974), the high chlorinity area corresponds to the mixing of ocean water with river water of $\delta^{18}\text{O} = -8.5$ ‰. For the domain with chlorinity below 7‰, the river end-member is characterised by $\delta^{18}\text{O} = -5.5$ ‰. A transitional zone exists between these two water masses. The water mass existing near the mouth which is located in the semi-enclosed basin of Marenne-Oleron results from the mixing of a 3 months old river water with $\delta^{18}\text{O} = -8.5$ ‰ with seawater.

Several factors may contribute towards the different isotope ratios encountered in river water. Some seasonal variations of the isotopic composition of precipitation reflect the parallel variation in the temperature of rain formation and sometimes in the relative humidity. The range of these long-term variations is

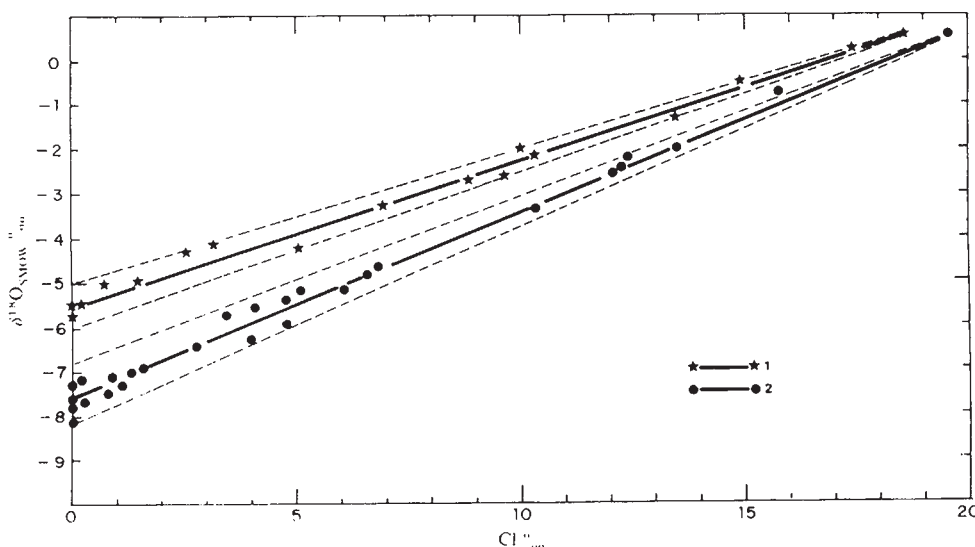


Fig. 2 ^{18}O content and chlorinity for: 1, Charente (22–26/10/1973); 2, Gironde (8–21/03/1978).

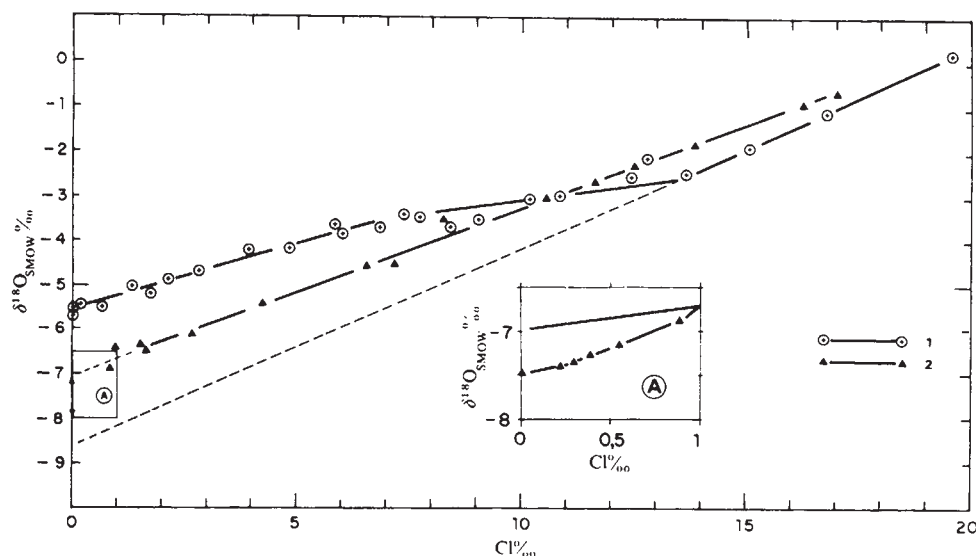


Fig. 3 $\delta^{18}\text{O}$ content and chlorinity for: 1, Charente (22-24/07/1974); 2, Gironde (21-26/10/1978).

important, especially in continental areas⁹. Once they reach the drainage area, these variations can be smoothed by processes such as evaporation, mixing of rain water with ground and superficial waters, buffering through lacustrine retention, and possibly through exchange with host rocks and dissolved gases. However, these isotope ratios generally remain a good tracer for hydrological studies, summer waters being enriched in ^{18}O in comparison with winter waters. On a short-term basis, the isotopic composition of river water can also change as a result of a piston effect due to storm waters which flush out the water from soils and shallow aquifers: such variations reaching several $\delta\%$ in ^{18}O have been observed in small alpine rivers¹⁰.

As well as varying with time, the $\delta^{18}\text{O}$ isotopic ratio can also change, for a given watershed, with the distance from the ocean and with altitude. The effect of altitude which has long been recognised as an important factor in isotopic hydrology is basically related to changes in temperature. Together with the 'continental effect'¹¹ it provides an efficient tool to differentiate waters of different origins.

This first assessment of ^{18}O distribution in estuaries suggests a new approach in the study of water mixing, making it possible to differentiate between water masses along the estuary, including those in areas of constant chlorinity such as in the Gironde estuary (see above). It is then obvious that the comparison of the theoretical distribution curve with the actual distribution of one given element by using a single river water end-member can be misleading. This is shown by the dissolved Ca distribution in the Charente estuary in July 1974. At that time, a definite excess of Ca in the middle estuary was attributed to a dissolution process¹². It is now clear from measurements of ^{18}O distribution that three water masses corresponding to river water of different chemical composition occurred along the estuary. Similarly, the PO_4^{3-} distribution, although biased by a severe pollution input, reflected the occurrence of the same water masses. In the Gironde estuary (March 1978) the river water samples which can be clearly defined through ^{18}O measurements, corresponds to a dissolved and particulate chemical composition very different from one sample to the other—for example, Zn, Cu and organic carbon, are respectively higher by factors of 1, 5 and 2 in the most downstream river sample (Cauwet *et al.* in preparation). More detailed analysis might provide a means of studying lateral mixing of different tributaries: in the Gironde estuary which receives two major rivers (Garonne and Dordogne rivers), the respective ^{18}O isotopic ratios during October 1978 survey were: ^{18}O -Garonne = $-7.75 \pm 0.27\%$ ($n = 17$) and ^{18}O -Dordogne = $-7.15 \pm 0.15\%$ ($n = 17$). In the case of Charente (1974) (Fig. 3) it was possible to estimate the estuarine water residence time.

The various freshwater components of estuaries might have different chemical compositions, so the classical comparison of

actual chemical distribution of one given element with that predicted from the mixing of one single river water with seawater, can complicate geochemical interpretation. Systematic ^{18}O analyses should be of value in deciphering these problems. A limiting factor of this approach is the variation of the river isotopic signal, but in this study the variation was negligible in six of the estuaries described in this paper.

We thank A. Filly for technical assistance. Financial support by CNRS is gratefully acknowledged.

Received 4 June; accepted 27 September 1979.

1. Meybeck, M., Hubert, P., Martin, J.-M. & Olive, Ph. in *Isotope Hydrology*, 523 (IAEA, Vienna, 1970).
2. Krouse, H. R. & Ross Mackay, J. *Can. J. Earth Sci.* **8**, 1107-1109 (1971).
3. Pritchard, D. W. *Proc. Am. Soc. Civil Engng* **81**, 711 (1955).
4. Epstein, S. & Mayeda, T. *Geochim. cosmochim. Acta* **4**, 213-214 (1953).
5. Duplessy, J.-C. thesis, Univ. Paris (1972).
6. Riley, J.-P. & Skirrow, G. *Chemical Oceanography* Vol. 1 (Academic, London, 1965).
7. Craig, H. & Gordon, L. J. *Stable Isotopes in Oceanographic Studies*, 9-130 (CNR, Spoleto, 1965).
8. Duplessy, J.-C. *C. r. heb. Séanc. Acad. Sci. Paris* **271**, 1075-1078 (1970).
9. IAEA-Environmental Isotope Data no. 5: *World Survey of Isotope Concentration in Precipitation* (IAEA, Vienna).
10. Blavoux, B. thesis, Univ. Paris (1978).
11. Dansgaard, W. *Tellus* **1**, 435-468 (1964).
12. Salvadori, F. thesis, Univ. Paris (1976).

Middle Pliocene climatic change in the western Mediterranean from faunal and oxygen isotopic trends

L. D. Keigwin Jr

Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881

R. C. Thunell

Department of Geology, University of South Carolina, Columbia, South Carolina 29208

The pelagic sedimentary sequences recovered by the Deep Sea Drilling Project (DSDP) from the Mediterranean are important because of their proximity to the classical shallow-water Neogene marine-type sections in Europe and because they may help correlations between the type sections and marine sequences outside the Mediterranean basin. We have studied the middle Pliocene (2.7-3.6 Myr ago) histories of surface-water temperature and oxygen isotopic composition at DSDP Site 132 in the Tyrrhenian Sea and here we compare these with another approach to estimating palaeotemperatures¹ based on the

transfer function technique². The record clearly shows a climatic cooling commencing between 3.2 and 3.0 Myr ago. An oxygen isotopic curve derived from the planktonic foraminiferan *Globigerinoides ruber* significantly correlates with a palaeotemperature record estimated from a transfer function palaeotemperature equation on planktonic foraminiferal data. Transfer functions may therefore be of value in interpreting palaeoclimatic history in sequences at least as old as the middle Pliocene.

Surface-water palaeotemperature estimates for Site 132 reveal relatively little temperature change before a cooling dated between about 3.1 and 3.2 Myr ago, followed by a slight warming over the Pliocene/Pleistocene boundary, and a cooling during the late Quaternary¹. Previous studies of the Mediterranean late Neogene based on DSDP sites include semi-quantitative studies^{3,4} and stable isotopic studies⁵ of planktonic Foraminifera.

The transfer function technique has previously only been used to quantify Quaternary climatic variation⁶. The approach relates the distribution of microfossil assemblages in surface sediments of the ocean to the hydrographic conditions in overlying surface waters. Past temperatures at a given location may then be estimated based on changes in the microfossil assemblages with time. In extending this method to pre-Quaternary assemblages, the most fundamental assumption is that the fossil species lived in similar conditions to those of the present-day species. As a working hypothesis, we assume that species in evolving lineages and species which are similar in morphology to present-day species also have similar environmental preferences. Similar assumptions were made in a study of late Cenozoic palaeoceanography of the Panama Basin⁷.

The middle Pliocene was chosen to isotopically test the reliability of pre-Quaternary palaeotemperature estimates using transfer functions for two reasons. First, middle Pliocene planktonic foraminiferal assemblages are significantly different from those of the Quaternary on which the transfer function is based. Thus, if our basic assumptions about morphologically similar species and evolving lineages of species are unjustified, the palaeotemperature estimates should become less accurate with increasing age. Second, the transfer function approach indicates a distinct cooling beginning about 3.2 Myr ago, a result which needed to be independently evaluated using stable isotopes.

Stable isotope stratigraphy using benthonic Foraminifera is based on correlating $\delta^{18}\text{O}$ variations which are inferred to

Table 1 Stable isotopic data (‰, B-1) for DSDP Site 132 samples

Sample (core- section, i.d. (cm))	Depth (m)	<i>Oridorsalis</i>		<i>G. ruber</i>	
		$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$
11-4 (49)	94.99	1.26	-2.50	-0.40	-1.04
11-5 (50)	96.50	1.40	-2.28	-0.94	-1.09
11-6 (50)	98.00	1.11	-2.29	-0.82	-0.74
12-1 (50)	99.50	1.41	-2.17	-0.38	-0.68
12-2 (50)	101.00	1.03	-1.75	-0.03	-0.55
		1.18	-1.95	-0.28	-0.39
12-3 (50)	102.50	0.82	-2.47	-0.45	-0.24
12-4 (50)	104.00	1.20	-2.05	-0.27	-0.37
12-5 (50)	105.50	1.19	-1.95	-0.33	-0.36
12-6 (50)	107.00	1.75	-2.41	-0.73	-0.52
13-1 (50)	108.50	0.92	-2.02	-0.55	-0.18
		1.00	-2.10		
13-2 (50)	110.00	1.08	—	-0.23	-0.35
				-0.30	-0.58
13-3 (50)	111.50	0.99	-2.16	-0.53	-0.35
13-4 (50)	113.00	0.96	-2.14	-1.43	-0.06
				-1.13	-0.22
13-5 (50)	114.50	1.05	-2.22	-1.20	-0.76
13-6 (50)	116.00	1.12	-2.17	-0.96	-0.24
14-1 (77)	117.77	1.09	-2.24	-0.96	-0.38
14-2 (41)	118.91	0.99	-2.11	-0.40	-0.55
14-3 (57)	120.57	0.93	-2.41	-1.09	-0.43
15-1 (53)	126.53	0.79	-1.94	-0.96	-0.02
		1.04	-2.01	-1.20	-0.01

largely reflect changes in continental ice volume⁸. During the Pliocene and early Quaternary the sill at the Gibraltar portal is thought to have been about 1 km deeper, based on ostracod assemblages⁹, and a stable isotopic study of the earliest Pliocene at DSDP Site 132 (ref. 10). Therefore, the western Mediterranean basin probably exchanged deeper intermediate waters with the Atlantic Ocean during the middle Pliocene and the $\delta^{18}\text{O}$ of benthonic Foraminifera at DSDP132 should largely reflect ice volume variability.

Standard procedures were used in the stable isotopic analyses of this study¹¹. Results are presented with respect to the powder standard B-1 because calibration of our working standard gas to PDB is incomplete at present. Friedman (personal communication to Shackleton¹²) has found the standard B-1 to be +0.29‰ with respect to PDB $\delta^{18}\text{O}$. Numerous analyses of B-1 give reproducibility (1 s.d.) of about ± 0.06 for $\delta^{18}\text{O}$. Precision is slightly less for foraminiferal samples. *Globigerinoides ruber* was chosen (from the size fraction 175–350 μm) for analysis because of its persistent abundance throughout the Pliocene at DSDP Site 132 and because it is a modern surface-dwelling species, thus providing a surface-water temperature message. The benthonic foraminiferal genus *Oridorsalis* was chosen for analysis because of its relative abundance and because in another measured Cenozoic section this genus showed no fractionation of $\delta^{18}\text{O}$ relative to *Uvigerina*¹¹. Likewise, Boersma and Shackleton¹² have reported no fractionation between *Uvigerina spinulosa* and *Oridorsalis umbonatus*. Unfortunately, *Oridorsalis* was rarely abundant enough to perform replicate analyses.

The chronological framework used in this study (Fig. 1) is based on previous calibration of planktonic foraminiferal events using a palaeomagnetic stratigraphy^{13–15}. The most useful events are the extinctions of *Globorotalia margaritae* at approximately 3.3 Myr ago and *Sphaeroidinellopsis* at 3.0 Myr ago.

Stable isotopic results are presented in Table 1 and Fig. 1. Simple statistics comparing temperature estimates and isotopic results for the intervals before and after evidence of climatic change are presented in Table 2. The $\delta^{18}\text{O}$ of both planktonic and benthonic Foraminifera exhibits a sharp permanent increase in the middle Pliocene. Faunal evidence¹ and stable isotopic results on *G. ruber* suggest climatic deterioration began at about 3.2 Myr whereas it is dated at about 3.1 Myr based on

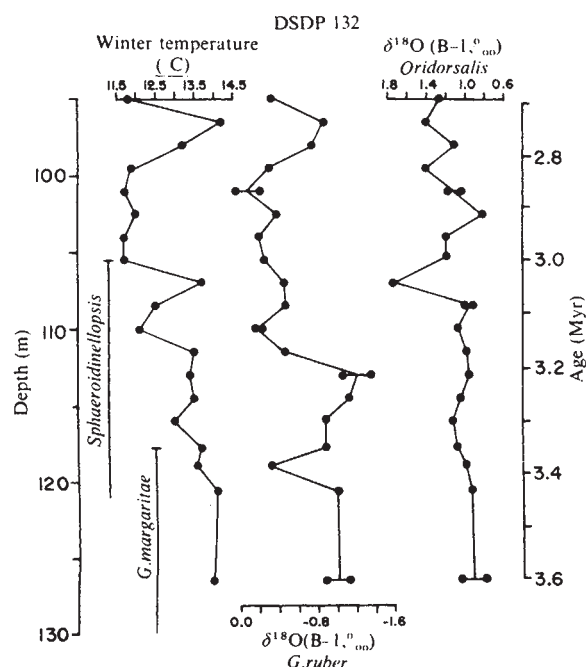


Fig. 1 Middle Pliocene palaeotemperature estimates (after Thunell¹) and oxygen isotopic results at DSDP Site 132.

Table 2 Simple statistical comparison of oxygen isotopic data and temperature estimates before and after middle Pliocene cooling

	Post-cooling			Pre-cooling		
	mean	s.d.	n	mean	s.d.	n
$\delta^{18}\text{O}$ <i>G. ruber</i>	-0.49	0.24	12	-1.00	0.29	7
$\delta^{18}\text{O}$ <i>Oridorsalis</i>	1.25	0.26	9	1.01	0.07	10
Winter T ($^{\circ}\text{C}$)*	12.4	0.89	11	13.6	0.35	8

*After Thunell¹.

Oridorsalis isotopic results (Fig. 1). Thus, evidence of pronounced cooling and ice volume increase occurs within an interval of about 0.1 Myr in the western basin of the Mediterranean.

The benthonic oxygen isotopic record between 3.1 and 3.6 Myr is highly stable and similar to other benthonic $\delta^{18}\text{O}$ records of the same age elsewhere. Thus, there is strong evidence that the Pliocene between 3.1 and 3.6 Myr ago was a time of relatively constant ice volume and bottom-water temperature^{11,16,17}. The fact that these Mediterranean results are similar to those outside the Mediterranean basin supports Benson's⁹ concept that deep, cold (psychrospheric) Pliocene ostracod assemblages in the Mediterranean indicate a deeper-water connection across the Gibraltar sill. At about 3.1 Myr the $\delta^{18}\text{O}$ of benthonic Foraminifera increases by about 0.8‰ and on average by 0.24‰ (significant at the 95% level) and becomes much more variable (Fig. 1, Table 2). The change at 3.1 Myr is interpreted as reflecting the first major increase in permanent Northern Hemisphere ice volume, supporting previous studies outside the Mediterranean^{11,18-22}. The detail of the DSDP Site 132 isotopic record, especially the sudden inferred ice growth between about 3.0 and 3.1 Myr is very similar to the records obtained at DSDP Sites 310 (ref. 11) and 397 (ref. 22). The increased variation in the benthonic $\delta^{18}\text{O}$ signal reflects an increase in climatic variation after 3.1 Myr as polar ice caps changed in volume. Evidence from glacial tills overlain by lavas^{18,19} and from deep-sea cores^{20,21} suggests that this first ice growth occurred between about 3.0 and 3.2 Myr ago, and is supported by our results.

The permanent isotopic enrichment recorded by *G. ruber* occurs at 3.2 Myr, predating that exhibited by *Oridorsalis* by about 0.1 Myr (Fig. 1). After about 3.2 Myr the $\delta^{18}\text{O}$ of *G. ruber* is enriched by an average of 0.51‰ (Table 2) which we interpret to reflect a decrease in surface-water temperature in addition to the compositional effect recorded by *Oridorsalis*. The oxygen isotopic record of *G. ruber* correlates well ($r = 0.79$, significant at the 99% level) with the winter palaeotemperatures calculated by Thunell¹, using the transfer function approach. A comparison of these curves (Fig. 1) shows that the sense of climatic change is the same but the amplitude is often different. Such differences in amplitude may result from the effects of evaporation and precipitation on $\delta^{18}\text{O}$. If we assume that the average isotopic enrichment of *Oridorsalis* after the onset of climatic cooling (0.24‰) represents only an ice volume change, and that the average enrichment of *G. ruber* (0.51‰) reflects an additional temperature effect, then the difference between the two (0.27‰) should be an estimate of the average surface-water oxygen isotopic change resulting from decreased temperatures after about 3.1 Myr ago. An isotopic change of about 0.27‰ represents about a 1 $^{\circ}\text{C}$ cooling, in good agreement with the average cooling estimated by faunal means (1.2 $^{\circ}\text{C}$; Table 2). Such agreement must be demonstrated for larger coolings and over longer time intervals. In general we believe that the transfer function approach is valuable for determining palaeotemperatures in Pliocene sequences, when the evolution of species lineages is taken into account.

The lag of about 0.1 Myr between the enrichment in the benthonic $\delta^{18}\text{O}$ record and the cooling indicated in both the palaeotemperature and planktonic $\delta^{18}\text{O}$ records indicates that a cooling of surface waters in the Mediterranean, and possibly

increased evaporation relative to precipitation, predated the build-up of permanent Northern Hemisphere ice sheets. The development of climatic cooling immediately before the initiation of Northern Hemisphere ice growth is also manifested as an intensification of abyssal circulation at approximately 3.3 Myr²³.

A comparison of the benthonic $\delta^{18}\text{O}$ record and the surface-water palaeotemperatures reveals that the initial growth of permanent ice occurred during a time of warm surface waters. A similar relationship has recently been observed by Ruddiman and McIntyre²⁴ in which they found periods of most rapid ice growth during the late Quaternary in the North Atlantic were associated with warm surface conditions. We speculate that warm surface waters may have provided a necessary source of moisture for the first permanent accumulations of Northern Hemisphere ice sheets.

We thank W. A. Berggren, B. H. Corliss, J. P. Kennett and T. S. Loutit for reviewing the manuscript. This work was supported by NSF grant OCE76-81489 (CENOP) and by a Woods Hole Oceanographic Institution postdoctoral fellowship to R.C.T.

Received 8 June; accepted 4 October 1979.

- Thunell, R. C. *Mar. Micropaleont.* **4**, 173-187 (1979).
- Imbrie, J. & Kipp, N. *Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 71-181 (Yale University Press, 1971).
- Cita, M. B. *et al. Init. Rep. DSDP* **13**, 1263-1339 (1973).
- Ciaranfi, N. and Cita, M. B. *Init. Rep. DSDP* **13**, 1387-1399 (1973).
- Longinelli, A. & Cita, M. B. *Init. Rep. DSDP* **13**, 1400-1404 (1973).
- CLIMAP Science **191**, 1131 (1976); CLIMAP Geol. Soc. Am. Mem. **145** (eds Cline, R. M. & Hays, J. D.) (1976).
- Keigwin, L. D. Jr. *Micropaleontology* **22**, 419-442 (1976).
- Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* **3**, 39-55 (1973).
- Benson, R. H. *The Mediterranean Sea* (ed. Stanley, D. J.) 63-73 (Dowden, Hutchinson and Ross, Stroudsburg, 1972).
- van Donk, J., Saito, T. & Shackleton, N. J. *Init. Rep. DSDP* **13**, 798-800 (1973).
- Keigwin, L. D. Jr. *Earth planet. Sci. Lett.* **45**, 361-382 (1979).
- Boersma, A. & Shackleton, N. J. *Init. Rep. DSDP* **39**, 911-924 (1977).
- Hays, J. D., Saito, T., Opdyke, N. D. & Burckle, L. H. *Bull. geol. Soc. Am.* **80**, 1481-1514 (1969).
- Saito, T., Burckle, L. H. & Hays, J. D. *Late Neogene Epoch Boundaries* (eds Saito, T. & Burckle, L. H.) 226-244 (Micropaleontology Press, New York, 1975).
- Ryan, W. B. F., Cita, M. B., Rawson, M., Burckle, L. H. & Saito, T. *Riv. ital. Paleont. Stratigr.* **80**, 631-688 (1974).
- Shackleton, N. J. & Kennett, J. P. *Init. Rep. DSDP* **29**, 801-807 (1975).
- Keigwin, L. D. Jr., Bender, M. L. & Kennett, J. P. *Science* **205**, 1386-1388 (1979).
- Curry, R. R. *Science* **154**, 770-771 (1966).
- McDougall, I. & Wensink, H. *Earth planet. Sci. Lett.* **1**, 232 (1966).
- Berggren, W. A. *Init. Rep. DSDP* **12** (1972).
- Shackleton, N. J. & Opdyke, N. D. *Nature* **270**, 216-219 (1977).
- Shackleton, N. J. & Cita, M. B. *Init. Rep. DSDP* **47**, 433-445 (1979).
- Ledbetter, M. T., Williams, D. F. & Ellwood, B. B. *Nature* **272**, 237-239 (1978).
- Ruddiman, W. & McIntyre, A. *Science* **204**, 173-175 (1979).

Nest of juveniles provides evidence of family structure among dinosaurs

John R. Horner

Department of Geological and Geophysical Sciences, Princeton University, Princeton, New Jersey 08544

Robert Makela

Rudyard High School, Rudyard, Montana 59540

One of the greatest mysteries associated with dinosaurs is the rarity of juveniles. With the notable exception of the Djadochta and Iren Dabasu Formations of Mongolia and the Two Medicine Formation of Montana, skeletal elements of very young dinosaurs are exceedingly uncommon¹⁻³, leaving a gap in the understanding of dinosaur social structures^{1,3,4}. The most popular explanation, based chiefly on negative evidence, is that the eggs and young were 'kept' in the uplands where they were rarely covered by sediments and as a result were destroyed by erosion². The discovery of fifteen 1-m long hadrosaurian ('duck-billed') skeletons together in a nest-like structure offers the first tangible evidence of the social behaviour of at least one group of dinosaurs. They were discovered by Marion Brandvold in terrestrial sediments of the Two Medicine Formation (Upper Cretaceous) near Choteau, Teton County, Montana.

The skeletons of 11 little hadrosaurs were found jumbled together in a fine-grained green mudstone that filled a bowl-shaped depression in a fine-grained brown mudstone (Fig. 1). The concave structure, oval in outline, was about 2 m in diameter at its weathered surface and extended about 0.75 m deep near its centre. Portions of four other individuals were scattered around the weathered surface, no more than 2 m away. Measurements of the skeletal elements, particularly the femora, indicate that all individuals were essentially the same size. The uncrushed femora vary between 120 and 130 mm long. Within the surrounding green mudstone were several minute fragments of eggshell and three terrestrial gastropods (family Polygyraeidae). Although it may have been an artefact created by differential weathering of the sediments, the concave structure was situated on the apex of a mound-shaped 'hill' approximately 3 m in diameter and about 1.5 m above the surrounding topography.

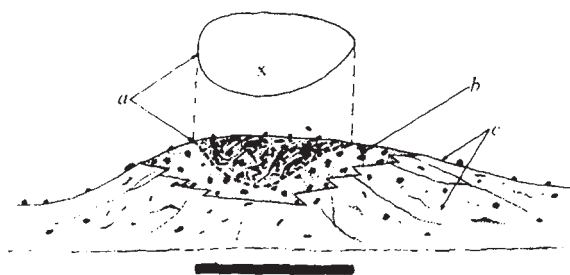


Fig. 1 Reconstruction of nest. *a*, Outline of green mudstone which contained the baby dinosaurs, eggshell and gastropods; *b*, brown mudstone that surrounded the green mudstone; *c*, weathered surface. (Scale bar, 2 m.)

The presence of several small skeletons, apparently all the same species and size, together with eggshell in an oval, concave structure, strongly suggests that the structure was a nest. The skeletons (Fig. 2) have several characteristics indicative of very young individuals. The sacral vertebrae are not fused; the neural arches are not fused to the vertebral centra; the proximal and distal ends of the limb bones are not well-formed, and the absence of the basicranial bones suggests that they were not ossified^{5,6}. Nevertheless, there is evidence that the young were capable of activity outside the nest. With the exception of the basicranial elements, all the skeletal elements present in an adult hadrosaur were already ossified, including the carpals and tarsals and the ossified tendons that run along the sides of the neural spines of the posterior dorsal, sacral and caudal vertebrae. The feet are somewhat large for the size of the animal, but the limb proportions are very similar to those of adult hadrosaurs, suggesting that very little ontogenetic change occurred in the postcranium during growth. Wear on the occlusal surfaces of the dental batteries indicates that the young had been feeding for some time. Some of the individual teeth are worn more than three-quarters their length. Another group of juvenile hadrosaurs found in the same general area, although several hundred metres from the nest, indicate that these nestlings are at least twice the size of hatchlings. Among the elements (PU 22432) are three femora averaging 69 mm in length, indicating that the animals were about 450 mm long. A considerable number of large eggshell fragments was found with the specimens.

The fact that 15 baby hadrosaurs had been feeding, and had stayed together for a period of time, indicates that some form of extended parental care was administered for, if the young were confined to the nest, food must have been brought to them. If the young had ventured out to forage for food, it is unlikely that they would have returned to the nest together without parental supervision.

Extended parental care is suggested by several other age-cohort associations of young dinosaurs in the same formation and area. Gilmore⁷ wrote that a mounted skeleton of the horned dinosaur *Brachyceratops montanensis* (USNM 7951) was made up of the bones of at least five individuals, all from the same deposit, all belonging to one species and all practically the same size. *Brachyceratops* is considered to be a juvenile of *Monoclonius*⁸ which averages about 4.2 m long, or slightly more than twice the length of the juveniles described by Gilmore. Of a second occurrence, Gilmore⁹ described a stratum full of scattered bones representing many individuals of a small hadrosaurian dinosaur. Measurements of the limb elements indicate that the individuals (USNM 16600) were about half the length of an average adult hadrosaur. The average length of the femora is 500 mm. Another group of hadrosaurs (USNM field no. Qu-3-35) collected by Gilmore consists of portions of at least three individuals each slightly less than half the length of an adult. The American Museum of Natural History has three individual hadrosaurs (AMNH 5358) which each have a femur length of about 450 mm.

All these groups of dinosaurs were collected from the Two Medicine Formation, no more than 0.5 km north of the nest described here. Of the more than 200 specimens from this formation now in the US National, American and Princeton Museums, more than 80% seem to pertain to individuals one twentieth to half the linear dimension of their adult counterparts. Eggshell fragments are the most common fossils in the formation and whole or partial eggs are not uncommon. The abundance of eggshell and juvenile hadrosaurs and ceratopsians in these sediments, as opposed to the scarcity of them in the adjacent Judith River (Oldman) Formation of Alberta^{2,10,11} and Montana (our observations), suggests that the depositional area of the Two Medicine Formation was a nesting ground for at least these two groups of ornithischian dinosaurs.

If the juvenile dinosaurs collected by the US National and American Museums from the Two Medicine Formation represent nestlings similar to those described here, parental care may have been extended until the young were about half the linear dimension of their adult counterparts, which suggests that the parent or parents spent considerable time with the young, or that the young grew very rapidly. As with birds, a rapidly growing dinosaur would have required a very high metabolic rate (endothermy), a considerable supply of food and a very efficient means to process the food. Efficient mastication seems to have been highly specialised among the hadrosaurs and ceratopsians, both of which possessed teeth able to pulverise and grind considerable quantities of food (? plant material). This may indicate that the other ornithischian dinosaurs, such as ankylosaurs and other ornithomimids which do not have complicated dental systems, either grew more slowly or had other means for efficient food processing.

Because of the immaturity of the juvenile dinosaurs and the ontogenetic changes which probably occurred in the skulls during growth, a specific identification of the 15 individuals from the nest may not be possible until more specimens of older individuals are found. Characteristics of the skulls, however, suggest that they are generically similar to a large skull (Fig. 2) found within 100 m of the nest. The large skull represents a new genus and species and, although it has some rather peculiar characteristics, is placed in the subfamily Hadrosaurinae.

Maiasaura (new genus)

Holotype: PU 22405, skull with partial right dentary and pre-dentary.

Referred specimens: PU 22400, skeletons of 15 individuals.

Horizon: Two Medicine Formation, Upper Cretaceous (Campanian).

Locality: 12 miles west of Choteau, Teton County, Montana, on the James and John Peebles ranch.

Collectors: J. R. Horner and R. Makela, 1978, for the Museum of Natural History, Princeton University.

Etymology: Maia from the Greek which means good mother, saura which means reptile (feminine).

Diagnosis: Wide elongate facial region. Short naris located on anterior end of snout with an extensive lateral facial region between the posterior end of the naris and the anterior end of the orbit. Short wide premaxillary bill with deflected border similar to that of lambeosaurines. Long shallow maxilla with an anterior maxillary process and anterior maxillary notch as present in hadrosaurines. Small, solid incipient crest above and between orbits, formed by the nasal and frontal bones. Nasals concave anterior to incipient crest. Quadratojugal process of the jugal long, thin and angling steeply upward to meet quadratojugal. Dentary teeth with long and narrow enamel crown. Prementary wide and very shallow with round, evenly spaced, denticulate processes around its anterior edge.

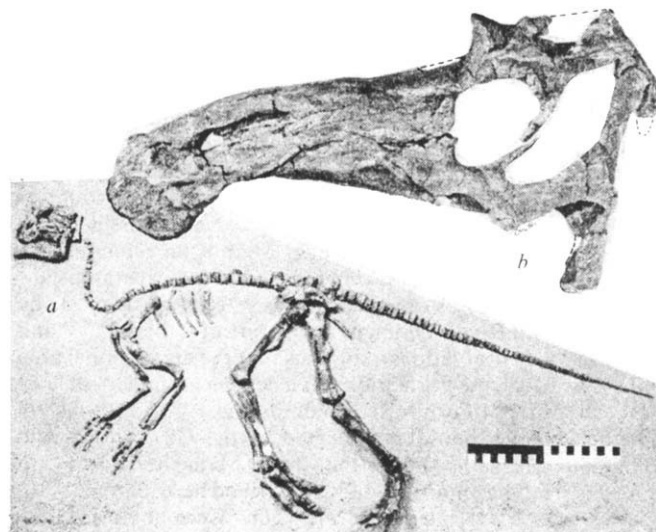


Fig. 2 *Maiasaura peeblesorum*. *a*, Incomplete reconstructed composite skeleton of individual from nest (PU 22400); *b* holotype skull (PU 22405). (Scale bar, 20 cm.)

Maiasaura peeblesorum (new species)

Specific characters same as generic characters.

Etymology: Specific name to honour the James and John Peebles families, owners of the land where the specimens were collected.

Discussion: The skulls of the referred juvenile specimens have short, wide deflected premaxillary bills, long shallow maxillae with anterior maxillary processes and notches and long, thin, steeply angling quadratojugal processes of the jugals. In all these characters they agree with the adult specimen. The juvenile skull elements are however disarticulated and in many instances crushed so that a positive identification cannot be made.

We thank M. Brandvold, D. Trexler and A. Luthin for their discoveries, the Peebles families for hospitality and D. Baird, D. Jones, P. Dodson and D. Weishampel for helpful discussions.

Received 22 June; accepted 21 September 1979.

1. Jepsen, G. L. *Am. Sci.* **52**, 227–247 (1964).
2. Sternberg, C. M. *Bull. Can. Dept. No. Affirs. Nat. Res.* **136**, 120–122 (1956).
3. Richmond, N. D. *J. Paleont.* **39**, 503–505 (1965).
4. Colbert, E. H. *The Year of the Dinosaur*, 1–171 (Scribner, New York 1977).
5. Kaye, J. M. & Russell, D. A. *J. Paleont.* **47**, 1, 91–93 (1973).
6. Langston, W. Jr *Fieldiana Geol. Mem.* **3**, 6, 313–360 (1960).
7. Gilmore, C. W. *Proc. U.S. nat. Mus.* **61**, 3, 1–4 (1922).
8. Colbert, E. H. *Evolution* **2**, 145–163 (1948).
9. Gilmore, C. W. *Exploration and Field Work for the Smithsonian Institution for 1929*, 7–12 (1929).
10. Dodson, P. *Palaeogeog. Palaeoclim. Palaeoecol.* **10**, 21–74 (1971).
11. Dodson, P. *Syst. Zool.* **24**, 37–54 (1975).

The Cossack skull and a dihybrid origin of the Australian Aborigines

L. Freedman

Department of Anatomy and Human Biology, University of Western Australia, Nedlands, Western Australia 6009

M. Lofgren

Department of Anthropology, Western Australian Museum, Francis Street, Perth, Western Australia 6000

Recent surveys of Australian prehistory^{1–4} reveal the interest and debate surrounding the evolutionary implications of the observed range in cranial morphology of modern Australian Aborigines and their predecessors. In spite of an earlier model postulating a trihybrid origin^{5,6}, a theory favouring a homogeneous colonising stock had gained some acceptance^{7,8}. Discoveries^{9,10} of the past decade raised doubts about the validity of the homogeneity concept and suggested an alternative two-population—gracile and robust—model^{11–13}. However, as all the material described was derived from a restricted area of South-East Australia, the possibility existed that the apparent dichotomy might reflect isolate variation or local adaptation¹⁴. We discuss here recently described human skeletal material from Cossack, Western Australia¹⁵ which has a demonstrable affinity with the postulated South-East Australian robust group, making local adaptation/population isolate explanations unlikely. The Cossack skull supports arguments favouring an early dihybrid origin with substantial change in Australian Aboriginal cranial morphology since the Pleistocene.

Discovered 3 km ESE of Cossack, Western Australia (Fig. 1), the material comprises most of a cranium and mandible together with fragments of the shafts of several limb bones. The skull was lying at the base of an eroding coastal dune and fragments of the long bones were scattered over the lower face of the dune. Direct dating of this individual is not feasible at present. However, the region's coastal features indicate that rising post-Pleistocene sea levels first reached the contemporary coastline around 6,500 yr BP¹⁶, thus apparently restricting the material's age to a period after that event.

Most areas of the skull are represented except for the base, the central portion of the face, and the left temporal, zygomatic and orbital regions; almost all the teeth are present. After cleaning and restoration, it was apparent that inward compression of the whole left side of the skull had occurred after death. However, the right side is relatively complete and undistorted, and some metrical observations are possible. On morphological criteria¹⁷, the individual is clearly male and was about 40 yr old at death.

The Cossack skull (Western Australian Museum no. A22684) includes a massive dolichocephalic calvaria with a large and slightly prognathous face (Fig. 2). The most striking feature is the markedly backward-sloping forehead. The supra-orbital margins are well developed and slope back strongly from the glabella to the large zygomatic trigones. Other features include some occipital bunning, an occipital torus, a clearly marked temporal line, a large supra-mastoid crest and a prominent malar tuberosity. The mandible is correspondingly large and robustly built, and the dentition shows heavy wear. The right 1st is missing and the alveolus completely resorbed, suggesting the possibility of tooth evulsion for cultural purposes as practised by recent Australian Aborigines.

Metrical comparison of the Cossack skull with a series of recent male Western Australian Aborigines¹⁸ reveals several

significant differences (Table 1). Cranial length is more than 5 standard deviations greater than the series mean. Although estimated breadth is slightly greater than the maximum Western Australian value, the cranial index of Cossack is comparable to the series lowest figure. Parietal size (bregma-lambda chord) is close to 3-standard deviations greater than the Western Australian mean. The Cossack skull's frontal curvature index is more than 5 standard deviations below the series minimum value. Cranial bone thicknesses are considerably greater than those of recent Australian Aborigines¹⁵. The most notable metrical mandibular comparison is of the corpus projective length, which yields a value more than 3 standard deviations greater than the series mean.

The features in which the Cossack skull differs from recent Western Australians are precisely those in which it resembles certain prehistoric South-East Australians (Table 1). Low frontal index and great length (the Cossack skull has the highest Australian values yet recorded) as well as general robusticity, highlighted by cranial vault bone thickness, are characteristic of the postulated robust group, including Talgai, Cohuna, Mossgiel and Kow Swamp. Comparison of lateral cranial contours¹⁵ reinforces the referral of the Cossack skull to this group.

The demonstrable affinity of the Cossack skull to the robust South-East Australian group indicates that this morphology was not a regional variant but continental in distribution. This newly



Fig. 2 Lateral view of the Cossack skull.

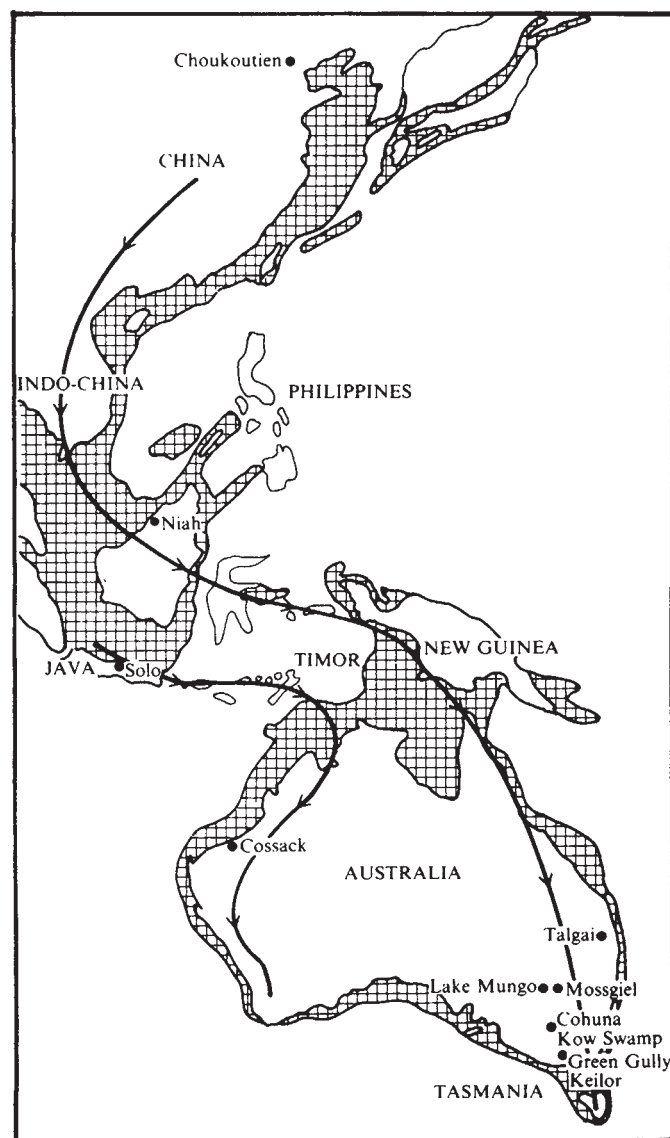


Fig. 1 Present and late Pleistocene (hatched) land areas. Fossil hominid sites and possible migration routes to Australia.

established range strengthens the evidence for a two-population occupation of Australia.

It is now generally accepted that human colonisation of the Australian continent first occurred at least 40,000 yr ago and probably earlier³. Fossil evidence in Australia of a more robust population extends from about 13,000 yr BP (Kow Swamp) to 6,000 yr BP (Mossgiel) and now possibly to even more recent times (Cossack). For the more gracile group the time span now ranges from 26,000 yr BP (Lake Mungo) to 13,000 yr BP (Keilor) to 6,000 yr BP (Green Gully)^{8,11,13}.

The two Asian stocks from which these populations could have been derived are: (1) a South-East Asian population, perhaps from Java and adjacent areas, and (2) a more northern population, possibly from southern China. These would be early *Homo sapiens* populations perhaps differing morphologically from each other as do the earlier Javanese and Chinese *Homo erectus* people. The more robust South-East Asian migrants, possibly morphologically related to the Solo (Ngandong) people¹⁹, could have taken a southerly route from Java by way of Timor into northwestern Australia and then down the west coast (Fig. 1). The more gracile population from southern China may have followed a more northerly course, perhaps passing through Indo-China, Borneo and New Guinea before arriving at the northeastern part of Australia. They would then have passed down the east coast and perhaps finally across the land bridge to Tasmania (Fig. 1). The 40,000-yr-old Niah cranium²⁰ from Borneo and the apparently more gracile appearance of the recent Tasmanians¹¹ may be the best evidence of this migration. These two early colonising Australian populations may have used different adaptive strategies. Their subsequent fusion might have been triggered initially by the climatic and faunal changes of the late Pleistocene but was only completed in recent times²².

Table 1 Selected metric comparisons

Measurement	Cossack ¹⁵	Male West. Aust. ¹⁸ (range)	Kow Swamp ²¹ (range)
Max. cranial length	220	169–202	190–214
Max. cranial breadth	(145)	117–143	128–150
Bregma-lambda chord	131	104–128	115–126
Cranial index	65.9	64.64–76.33	(66.67)–72.39
Frontal curvature index	12.6	18.58–25.66	12.71–16.12
Mandibular corpus projective length	98	71–95	

Measurements are given in mm.

Rapid advances have been made in the study of the prehistory of the Australian Aborigines over the past 10 yr. Syntheses of the new material and concepts^{1-4,11-13} are now bringing closer an understanding of the people who in the latter part of the Pleistocene colonised one of the last two major unoccupied land masses.

Received 10 July; accepted 26 September 1979.

1. Mulvaney, D. J. *The Prehistory of Australia* (Penguin, Melbourne, 1975).
2. Hallam, S. J. *Quat. Res.* **8**, 128-148 (1977).
3. Jones, R. J. *hum. Evolut.* **6**, 353-361 (1977).
4. White, J. P. & O'Connell, J. F. *Science* **203**, 21-28 (1979).
5. Birdsell, J. *Rec. Queen Vic. Museum* **2**, 105-122 (1949).
6. Birdsell, J. *Arch. phys. Anth. Oceania* **2**, 100-155 (1967).
7. Abbie, A. A. *The Original Australians* (Reed, Wellington, 1969).
8. Macintosh, N. W. G. Larnach, S. L. in *The Origin of the Australians* (eds Kirk, R. L. & Thorne, A. G.) 113-126 (Australian Institute of Aboriginal Studies, Canberra, 1976).
9. Bowler, J. M., Jones, R., Allen, H. & Thorne, A. G. *World Arch.* **2**, 39-60 (1970).
10. Thorne, A. G. & Macumber, P. G. *Nature* **233**, 316-319 (1972).
11. Howells, W. W. *The Pacific Islanders* (Reed, Wellington, 1973).
12. Thorne, A. G. in *Sunda and Sahul: Prehistoric Studies in Southeast Asia, Melanesia and Australia* (eds Allen, J., Golson, J. & Jones, R.) 187-204 (Academic, New York, 1977).
13. Thorne, A. G. & Wilson, S. R. *J. hum. Evolut.* **6**, 393-402 (1977).
14. Wright, R. V. S. in *The Origin of the Australians* (eds Kirk, R. L. & Thorne, A. G.) 265-276 (Australian Institute of Aboriginal Studies, Canberra, 1976).
15. Freedman, L. & Lofgren, M. J. *hum. Evolut.* **8**, 283-299 (1979).
16. Chappell, J. & Thom, B. G. in *Sunda and Sahul: Prehistoric Studies in Southeast Asia, Melanesia and Australia* (eds Allen, J., Golson, J. & Jones, R.) 275-291 (Academic, New York, 1977).
17. Larnach, S. L. & Freedman, L. *Rec. Aust. Mus.* **26**, 295-308 (1964).
18. Margetts, B. M. & Freedman, L. *Rec. West. Aust. Mus.* **6**, 63-105 (1977).
19. Weidenreich, F. *Anth. Pap. Am. Mus. nat. Hist.* **43**(3) (1951).
20. Brothwell, D. R. *Sarawak Mus. J.* **9**, 323-349 (1960).
21. Thorne, A. G. in *The Origin of the Australians* (eds Kirk, R. L. & Thorne, A. G.) 95-111 (Australian Institute of Aboriginal Studies, Canberra, 1976).
22. Bowler, J., Hope, G., Jennings, J., Singh, G. & Walker, D. *Quat. Res.* **6**, 359-394 (1976).

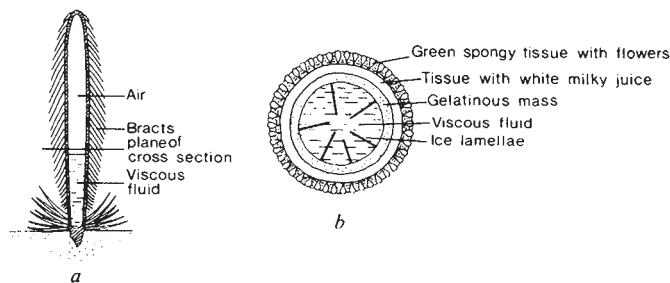


Fig. 1 Longitudinal section (a) and cross section (b) of *Lobelia telekii*.

during the day by means of thermocouples mounted in fixed locations. Temperatures recorded are shown in Fig. 2. After sunrise the temperature in the flower rose to several degrees above that of the air, indicating that the bracts are also important for absorbing radiant heat from the Sun². The absorbed heat also gradually leads to an increase in the temperature of the central fluid. As the air temperature dropped in the afternoon, the temperature in the central fluid lagged behind. In contrast to the temperature in the air, the temperature in the flower and in the central fluid did not drop below zero during the night; the temperature in these parts of the plants levelled out at zero and stayed there until sunrise.

The time lag between the drop in air temperature and the fall in temperatures measured in the plant is mainly due to the high heat capacity of the central fluid. This time lag, as well as the stabilisation of the temperature of the plant at zero, imply that the flowers are thereby protected from exposure to the low and possibly injurious air temperature.

When the inflorescence of a *L. telekii* plant was cut just above the surface level of the central fluid at sunrise, the central fluid was found to contain ice, in the form of vertical thin lamellae, which protruded radially into the central fluid from the wall of the inflorescence, as indicated in Fig. 1b. The amount of ice was estimated at ~2% of the fluid volume. Liberation of the heat of fusion of the frozen water leads to a stabilisation of the temperature at the melting point of the unfrozen fraction of the central fluid. Thus the formation of ice in the central fluid and the concomitant liberation of the heat of fusion form the physical basis for the stabilisation of fluid and flower temperature at about 0 °C during the night. The temperature of the partially frozen central fluid was 0.1 °C, indicating that in spite of its viscous character, the central fluid has a very low osmotic activity. This will ensure maximal thermal protection of the plant by leading to a stabilisation of the temperature of the frozen central fluid at the highest possible subzero temperature.

The freezing of the central fluid will in turn also have a thermally buffering effect on the air-filled compartment above

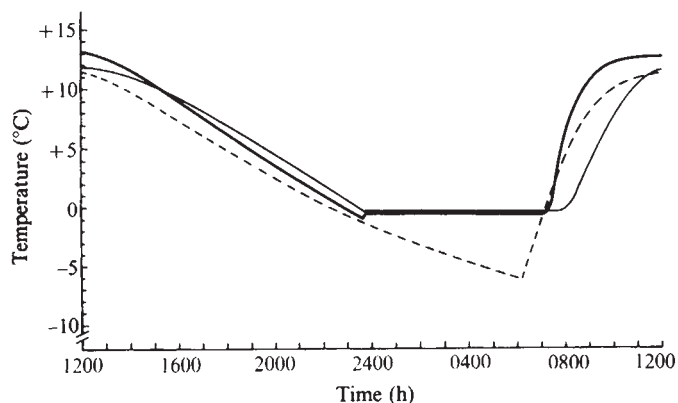


Fig. 2 Temperature variations of the air (dashed line), the central part of a flower (heavy line) and the central fluid in the inflorescence (thin line) of a *Lobelia telekii* plant.

Thermal buffering in Afro-alpine plants due to nucleating agent-induced water freezing

John O. Krog*, Karl Erik Zachariassen†, Bjørn Larsen‡ & Olav Smidsrød‡

*Institute of Zoophysiology, University of Oslo, Blindern, Oslo 3, Norway

†Institute of Zoology, The University of Trondheim, Rosenborg, 7000 Trondheim

‡Institute of Marine Biochemistry, The University of Trondheim, 7034 Trondheim—NTH, Norway

Plants in the alpine zone of Mount Kenya are exposed throughout the year to environmental temperatures varying from about -10 to +10 °C (ref. 1). In contrast to plants in Arctic and temperate regions, which are in a state of suspended growth during the cold season, these plants have to combine growth with tolerance to low environmental temperatures. It has been suggested that flowers of the Afro-alpine plant *Lobelia telekii* gain thermal protection from the long, thin bracts which hang down at the outside of the inflorescence, and which seem to lower the radiative heat loss of the plants at night¹. We report here that vital tissues of *L. telekii* are also protected by the heat of fusion of water, which is present in the central part of the inflorescence, and that freezing of this water is ensured by the presence of nucleating agents, which induce freezing at a few degrees below zero.

The inflorescence of *L. telekii*, which may reach a height of 2 m, is cylindrical and hollow, with an inner diameter of 5-8 cm (Fig. 1a). The central cavity contains a slightly viscous fluid, which is apparently produced by the plant. The fluid level within the plant reaches more than 0.5 m above the ground, and several litres may be drained from each inflorescence. The temperature of the air and in the central part of a flower and that of the central fluid in the inflorescence of a *L. telekii* plant were measured

the central fluid (Fig. 1a). As the air is trapped inside the inflorescence and cannot escape, it will circulate inside the upper part of the inflorescence and cause internal heating at the top of the plant. Thus, the heat of fusion liberated in the central fluid will contribute not only to the thermal protection of those parts of the plant which surround the central fluid, but also to the whole inflorescence.

Based on these observations, it seems to be of importance to the survival of the plant to induce freezing in the central fluid at the highest possible subzero temperature. Freezing at high temperatures and prevention of supercooling are known to be affected by the presence of so called nucleating agents. A search for such agents in the internal fluids from the plant was initiated, using a method described by Zachariassen and Hammel³. Samples of central fluid (0.25 µl) were transferred to 5-µl samples of 0.9% NaCl solution, and the supercooling point of the mixed samples was measured. The supercooling point of four parallel samples was -4.6 ± 1.3 °C ($\pm$ s.d.), whereas samples of undiluted NaCl solution supercooled to -15 to -20 °C. This revealed that the central fluid of the inflorescence of *L. telekii* plants must contain nucleating agents, which will induce freezing at as high temperatures as possible.

The nucleating effect of the central fluid was still intact following heating of the fluid to $+100$ °C for 5 min. Investigations of the chemical composition of the central fluid revealed that the fluid contains about 15 mg ml^{-1} of carbohydrates, as determined by the phenol sulphuric acid colour method⁴, using

glucose as standard. The content of protein was found to be about 0.15 mg ml^{-1} by the FC analysis⁵, using bovine serum albumin as a standard. This low protein value agreed with analysis for nitrogen on a Carlo Erba elemental analyser Model 1104. A fraction of the organic material was non-dialysable, indicating that the central fluid contains a high molecular weight polysaccharide as one of the components. The high heat stability of the nucleating agents, combined with the quantitative domination of carbohydrates in the fluid, might indicate that the nucleating agents are carbohydrates.

After a standard hydrolysis ($1 \text{ M H}_2\text{SO}_4$, $+100$ °C, 5 h), the monomeric sugars in a yield of about 30% were separated as glycol hexoacetates by gas chromatography. By co-chromatography, the main glycolols were shown to be iditol, glucitol and mannitol. The carbohydrates gave a strong positive reaction in a test for keto sugars⁶. Work to identify the sugars and to elucidate the constitution of the polysaccharides is being carried out.

This study was supported by a grant from the Norwegian Research Council for Science and the Humanities.

Received 22 June; accepted 31 August 1979.

1. Coe, M. J. *The Ecology of the Alpine Zone of Mount Kenya* (W. Junk, The Hague, 1967).
2. Krog, J. *Physiologia Pl.* **8**, 836–839 (1955).
3. Zachariassen, K. E. & Hammel, H. T. *Nature* **262**, 285–287 (1976).
4. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. *Analyt. Chem.* **28**, 350–356 (1956).
5. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).
6. Yaphe, W. & Arsenault, G. P. *Analyt. Biochem.* **13**, 143–148 (1965).

Melatonin, the pineal gland and human puberty

R. E. Silman, R. M. Leone & R. J. L. Hooper

Departments of Reproductive Physiology and Chemical Pathology, St Bartholomew's Hospital Medical College, London EC1, UK

M. A. Preece

Department of Growth and Development, Institute of Child Health, London EC1, UK

Animal experiments have suggested that the pineal gland produces an anti-gonadotropic hormone. The hamster, for example, undergoes reproductive collapse when kept in short-day periods, an effect which is abolished by pinealectomy¹. Although there is little direct evidence about the endocrine role of the pineal gland in man, it has been noted that tumours of the pineal gland in young boys are associated with precocious puberty and the human pineal gland has been suggested to produce a substance that holds sexual maturation in check². This observation has been extended by Kitay³, who has shown that destructive tumours are associated with precocious puberty whereas hyperactive tumours are associated with delayed puberty. However, no studies have described any change of pineal function with normal puberty. Because two pineal indoles, melatonin⁴ and methoxytryptophol⁵, have been shown to be antigonadotropic when administered to animals^{6–10}, we have now measured them in schoolchildren. Our findings show that in young boys there is an abrupt fall in the concentration of melatonin with advancing development suggesting that it may play an important physiological role in the control of human puberty.

We studied 51 healthy boys and girls between the ages of 11½ and 14 yr; all attended a normal secondary school in Somerset, UK and blood was taken between 1100 and 1300 hr. At the time of sampling each child was assigned to one of five maturity stages¹¹ according to genitalia or breast development. Some of the children were studied on up to four occasions such that an overall total of 101 blood samples was taken. In addition to

pineal hormones, serum luteinising hormone (LH), follicle stimulating hormone (FSH), testosterone and oestradiol were measured by radioimmunoassay. Melatonin and methoxytryptophol were assayed by gas chromatography–mass spectrometry (GCMS)^{12,13} (Fig. 1). The sensitivity of the assay was assured by single ion monitoring at m/e 232 because this was the most abundant fragment ion in the mass spectra of melatonin and methoxytryptophol. It was also a fragment characteristic of the methoxyindole nucleus and therefore contributed to the specificity of the assay. Specificity was further enhanced by increasing the resolution to 2,000 so that the more precise mass 232.12 was monitored. Selected samples from the schoolchildren which gave high values for melatonin using the routine assay (monitored at m/e 232) were validated by being re-assayed at m/e 245 and m/e 304 and results were consistent with those previously obtained. The ratio of m/e 232 to m/e 245 was 1.23 ± 0.16 s.d. (synthetic melatonin 1.21 ± 0.04).

The concentration of methoxytryptophol ranged from <4 to 92 pg ml^{-1} in the boys and from <2 to 400 pg ml^{-1} in the girls and did not show a sex distribution or any change with development. The concentration of melatonin in the schoolgirls ranged from <5 to 280 pg ml^{-1} and did not change with development. In contrast, the concentration of melatonin in the boys which ranged from <10 to $2,300 \text{ pg ml}^{-1}$ showed a marked change during adolescent development (Fig. 2). The means and 95% confidence limits were calculated from log transformed data by mixed longitudinal methods described by Tanner¹⁴. At genitalia stage 1 the mean value of melatonin was 218 pg ml^{-1} (95% confidence limits 509–93, $n = 13$). At stages 2, 3 and 4 the mean values of melatonin were 30 pg ml^{-1} (128–18, $n = 28$), 17 pg ml^{-1} (24–13, $n = 15$) and 18 pg ml^{-1} (32–11, $n = 11$). At stage 5 there were only two samples from the school population and to these were added the daytime values from normal adults, making a mean of 16 pg ml^{-1} (11–21, $n = 18$). The fall in concentration of melatonin from stage 1 to stage 2 was highly significant ($P < 0.001$), but no significant changes occurred thereafter.

When the concentration of melatonin was tabulated against the concentration of LH assayed in the same samples (Fig. 3), the mean value of melatonin decreased from 287 pg ml^{-1} to 24 pg ml^{-1} when the concentration of LH increased from

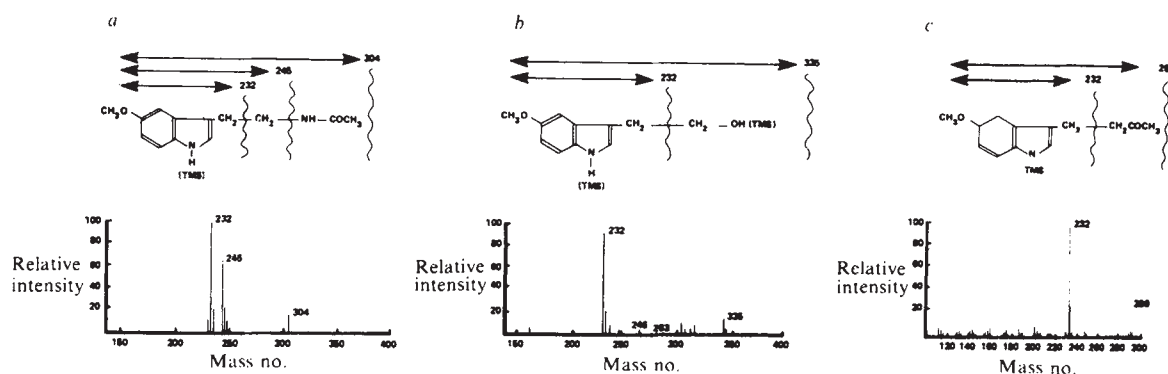


Fig. 1 Molecular structures and partial normalised mass spectra of TMS derivatised melatonin (a), methoxytryptophol (b) and the internal standard [3-(butan-3'-one)5-methoxyindole] (c) are illustrated. Internal standard (50 ng) was added to 1 ml of plasma or serum sample and vortex mixed with 3 ml of chloroform. The organic phase was separated and evaporated to dryness. Before assay the dried extract was reconstituted in 10 μ l of silylation reagent. Portions of 5 μ l were injected on to GCMS. Methoxytryptophol entered the ion source at 2 min 15 s, the internal standard at 2 min 40 s and melatonin at 4 min 40 s. The magnet and electrostatic analyser were tuned to allow only the passage of ions of mass 232 (strictly m/e 232). Each sample was quantified by determining the ratio of the peak height of endogenous melatonin or methoxytryptophol to the peak height of the internal standard and calculating the concentration from a standard curve.

undetectable values to values measurable but still below 1 IU l^{-1} . This fall in concentration of melatonin was highly significant ($P < 0.001$). The analysis of the fall in concentration of melatonin relative to increasing levels of FSH or testosterone showed the same pattern but the association was less striking.

We have reported a limited study and further work is required, especially in relating these findings to circadian changes. However, it is already clear that, using the same GCMS assay for melatonin as reported here, the levels of melatonin found in most samples from the prepubertal schoolboys exceed anything measured in the day or night from normal adult volunteers who have been monitored in a sleep laboratory (P. E. Mullen, unpublished observations). Also, studies on the circadian values of melatonin in pubertal boys show values which are no different from those of the mature adult¹⁵. However, ethical considerations have prevented investigation of prepubertal boys in the sleep laboratory where it would have been valuable to relate the circadian changes of melatonin to the first night time increases in gonadotropin secretion which herald puberty¹⁶.

Nonetheless our results clearly show that the circulating level of melatonin drops significantly in schoolboys before the first

physical signs of puberty have become apparent and before the increase in gonadotropins and testosterone which accompanies puberty. Animal experimentation had indicated that melatonin can act on the hypothalamus and pituitary to suppress the release of LH and FSH^{6,7}. Melatonin may also act directly on the gonads to suppress ovarian and testicular function⁸. Thus it is possible that the drop in melatonin, which we have observed, is part of the process which initiates the physical and endocrine changes of puberty.

A major unsolved problem is whether the changes in melatonin secretion also occur in young girls. We have been unable to show any fall in the concentration of melatonin in our schoolgirl population and this may indicate that there is a sex difference in pineal endocrinology. Alternatively there may be an age

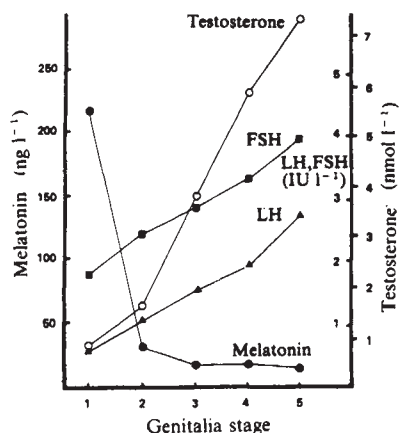


Fig. 2 Mean concentration (calculated from log transformed data) of melatonin (●), FSH (■), LH (▲), and testosterone (○) in serum samples from schoolboys. The values are grouped for each stage of puberty at which they were obtained. Stages: 1, testes, scrotum and penis the same size and proportion as in early childhood; 2, enlargement of testis and scrotum, change in texture and some reddening of scrotal skin; 3, further growth of penis, mainly in length but some increase in breadth; 4, further enlargement of penis in length and breadth and development of glans; 5, genitalia adult in size and shape.

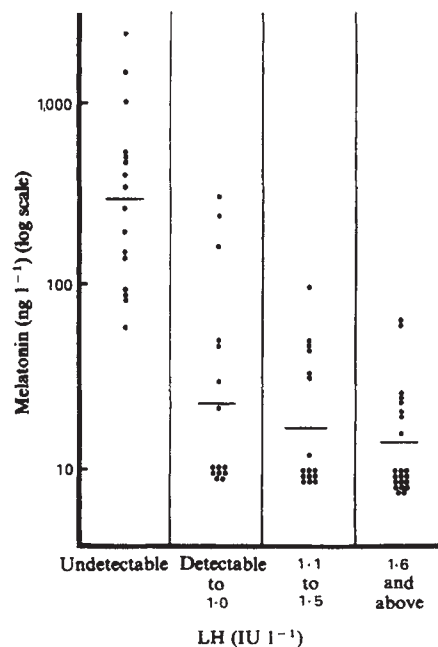


Fig. 3 The concentration of melatonin has been plotted against the concentration of LH in the same sample and the values of LH shown as four groups. a, LH undetectable, mean concentration of melatonin 287 $pg\ ml^{-1}$ (95% confidence limits 494–167, $n = 16$); b, LH detectable to 1.0 IU l^{-1} , melatonin 24 $pg\ ml^{-1}$ (48–12, $n = 15$); c, LH 1.1–1.5 IU l^{-1} , melatonin 17 $pg\ ml^{-1}$ (24–11, $n = 16$); d, LH 1.6 and above IU l^{-1} , melatonin 14 $pg\ ml^{-1}$ (21–10, $n = 22$). The mean concentration of melatonin in each group is shown by the horizontal bar. The limit of detection for the GCMS assay of melatonin was approximately 10 $pg\ ml^{-1}$.

difference insofar as the girls have matured earlier than the boys. Thus a drop in the concentration of melatonin may have occurred in the girls but at a younger age than we were able to obtain in our school sample.

Finally, it is curious that the fall in concentration of melatonin is probably evident without the need to use expensive technology. Melatonin is the most potent lightener of melanocytes¹⁷ and has been shown to lighten mammalian hair¹⁸ and human skin¹⁹. Because children are fairer skinned and lighter haired when they are young and become darker as they grow older, it is likely that the fall in concentration of melatonin is the stimulus for such colour changes, giving visible sign of the early endocrine changes of puberty.

We thank Janet Baines-Preece and Dr N. C. Cameron for assessing the stages of puberty in the schoolchildren and Dr K. Nicol and S. Brown for the assay of gonadotropins and gonadal steroids. We also thank the children and staff at Hollyrood School, Chard, Somerset, for participating in the study. This work was supported by a grant from the Wellcome Trust and is part of an interhospital, interdisciplinary project with Drs P. Mullen and Ivor Smith.

Received 9 August; accepted 26 September 1979.

- Hoffman, R. A. & Reiter, R. J. *Science* **142**, 1609 (1965).
- Heubner, O. *Di. med. Wschr.* **24**, 214 (1899).
- Kitay, J. I. *Clin. Endocr.* **14**, 622 (1954).
- Lerner, A. B., Case, J. D. & Heinzelman, R. V. *J. Am. chem. Soc.* **81**, 6084 (1959).
- McIsaac, W. M., Farrell, G., Taborsky, R. G., Taylor, A. N. *Science* **148**, 102 (1965).
- Tamarkin, L., Hollister, C. W., Lefebvre, N. G. & Goldman, B. D. *Science* **198**, 953 (1977).
- Martin, J. E., Engel, J. N. & Klein, D. C. *Endocrinology* **100**, 675 (1977).
- MacPhee, A. A., Cole, F. E. & Rice, B. F. *J. clin. Endocr. Metab.* **40**, 688 (1975).
- McIsaac, W. M., Taborsky, R. G. & Farrell, G. *Science* **145**, 63 (1964).
- Hipkin, L. J. *J. Endocr.* **48**, 287 (1970).
- Tanner, J. M. in *Growth at Adolescence*, 2nd edn (Blackwell, Oxford and Thomas, Springfield, 1962).
- Leone, R. M. *et al. J. Endocr.* **82**, 243 (1979).
- Leone, R. M. *et al. Prog. Brain Res.* (in the press).
- Tanner, J. M. *Hum. Biol.* **23**, 93 (1951).
- Fevre, M., Segel, T., Marks, J. F. & Boyar, R. M. *J. clin. Endocr. Metab.* **47**, 1383 (1978).
- Boyar, R. *et al. New Engl. J. Med.* **287**, 582 (1972).
- Lerner, A. B. & Wright, R. M. *Methods biochem. Analysis* **8**, 295 (1960).
- Hoffman, K. J. *comp. Physiol.* **85**, 267 (1973).
- Nordlund, J. J., & Lerner, A. B. *J. clin. Endocr. Metab.* **45**, 768 (1977).

Specific distribution of metabolic alterations in cerebral cortex following apomorphine administration

James McCulloch*, Helen E. Savaki,
Mailis C. McCulloch* & Louis Sokoloff

Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, Maryland 20205

*University of Glasgow, UK

The topographic distribution of dopaminergic receptors in the cerebral cortex closely parallels that of the dopaminergic innervation¹⁻⁶. In the rat, dopaminergic axons which originate in the mesencephalon are confined to a few discrete regions of the neocortex—anterior cingulate cortex, entorhinal cortex, frontal cortex (particularly anteromedial and supragenual areas) and the transitional zone between the neocortex and the pyriform cortex¹⁻⁴. Moreover, biochemical examinations of processes generally considered to be indicative of dopaminergic neurotransmission—neuronal uptake of labelled dopamine or dopamine-activation of adenylate cyclase activity^{5,6}—have confirmed a highly restricted locus of action of dopaminergic systems in the cerebral cortex. We describe here data obtained using the 2-deoxyglucose technique⁷ in conjunction with conventional neuropharmacological techniques, suggesting that the influence of dopaminergic systems on cortical function extends beyond the known confines of the mesocortical dopaminergic system.

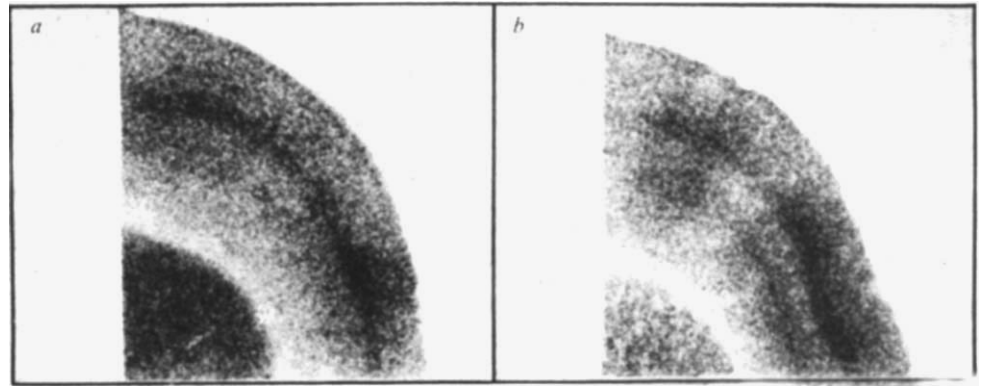
The technique using ¹⁴C-labelled 2-deoxyglucose for the measurement of the rates of local cerebral glucose utilisation⁷ offers a novel approach to study the pharmacology and physiology of the central nervous system (CNS). The energy requirements of cerebral tissue are derived mainly from the metabolism of glucose, and alterations in functional activity of any region of the brain are associated with changes in its energy demands⁸. The ¹⁴C-labelled 2-deoxyglucose technique, which involves the use of quantitative autoradiography to localise radioactivity in the CNS, makes possible the comprehensive assessment of functional alterations throughout the CNS with a degree of localisation impossible with many other approaches⁸. Details of this method have been reported previously⁷. Our experiments were performed on conscious, restrained, male Sprague-Dawley rats weighing approximately 350 g, in which a femoral artery and vein had been catheterised at least 2 h previously. Local rates of glucose utilisation were calculated, by means of the operational equation of the method⁷, from the local concentrations of ¹⁴C in cerebral tissue (determined by quantitative autoradiography) and the time course of the arterial plasma ¹⁴C-deoxyglucose and glucose concentrations. Drugs were administered intravenously; apomorphine was injected 10 min and haloperidol 25 min before the pulse of ¹⁴C-deoxyglucose. Administration of the drugs was associated with gross behavioural alterations. With apomorphine, the pattern was characterised by sniffing, licking and movement of the upper body, all of which were replaced at higher concentrations by almost continuous gnawing. With haloperidol, all animals displayed lesser degrees of spontaneous movement, culminating, at doses of 0.1 mg per kg or greater, in a dose-dependent cataleptic state.

Administration of apomorphine resulted in statistically significant dose-dependent alterations in the rates of glucose utilisation in several regions in the CNS in which dopamine is suspected to play a major functional role. For example, glucose utilisation was increased in the caudate nucleus by 29%, in the globus pallidus by 41%, in the substantia nigra zona compacta by 41%, and in the substantia nigra zona reticulata by 76% after the administration of apomorphine (0.5 mg per kg). The effects of apomorphine in the extrapyramidal and other systems will be described in detail elsewhere. We report here only the effects observed in the cerebral cortex, which suggest that the action of apomorphine, the most widely used dopaminergic receptor agonist, on glucose utilisation in the cerebral cortex of the conscious rat is widespread and complex, in spite of the limited extent of cerebral cortical dopaminergic receptors and innervations.

The rate of glucose utilisation normally varies relatively little in different areas of the cerebral cortex, except in the primary auditory cortex where it is markedly greater than in other cortical regions⁷. Metabolic activity is also fairly uniform throughout the depths of the cortex, with the exception of cortical layer IV in which glucose utilisation is approximately 50% greater than in other cortical laminae (Figs 1 and 2).

Apomorphine effected dose-dependent reductions of glucose utilisation in the anterior cingulate cortex, a region innervated by dopaminergic neurones¹⁻⁴ and possessing dopaminergic receptor mechanisms⁵ (Fig. 2a). In contrast, in the dorsolateral frontal cortex and the sensory motor cortex, regions not generally recognised as primary dopaminergic projection areas, apomorphine caused significant increases in glucose utilisation (Fig. 2b). In the frontal and sensory motor cortices, the most marked elevations of glucose utilisation were observed in the laminae corresponding to layer VI and the deeper portions of layer V (Fig. 2c). Thus, in apomorphine-treated animals this layer, in addition to layer IV, could readily be distinguished on the autoradiographs (Fig. 1). Quantitative determination of the rates of glucose utilisation confirmed the changes seen in the autoradiographs (Fig. 2). Statistically significant increases in the sensory motor cortex were observed even with the smallest dose of apomorphine (0.15 mg per kg) used (Fig. 2). In addition to this laminar pattern of metabolic activation in the dorsolateral

Fig. 1 Autoradiograms of brain sections illustrating the action of apomorphine on the pattern of glucose utilisation in sensory motor cortex of a control animal (*a*) and an animal which had received apomorphine (*b*) (1.5 mg per kg).



frontal and sensory motor cortices, alternating columns of increased and decreased glucose utilisation traversing the thickness of the cortex in these same regions were readily discernible in the autoradiographs (Fig. 1). The columnar and laminar alterations in cortical glucose utilisation decreased progressively in a rostro-caudal direction. In a number of major cortical regions, such as the posterior parietal cortex (Fig. 2*d*), the primary visual cortex and the primary auditory cortex, no alterations in metabolic activity were observed after administration of apomorphine. All the effects of apomorphine in all the cortical areas examined could be prevented by prior administration of haloperidol (0.1 mg per kg).

Previous investigations of the influence of dopamine-agonists and antagonists on cortical function have concentrated on the

mesocortical dopaminergic system (particularly, the anterior cingulate cortex and the anteromedial frontal cortex). It is within these small cortical regions that: (1) the dopaminergic nerve terminals originating from the A9 and A10 cell groups of the mesencephalon are found¹⁻⁴; (2) dopamine-sensitive adenylyl cyclase has been characterised⁵; (3) specific uptake mechanisms for dopamine have been identified⁶; and (4) cell groupings in which the firing rate is sensitive primarily to dopamine have been localised⁹. Thus, in view of the close coupling between local functional activity and glucose utilisation in the CNS⁸, the observation that glucose utilisation in the anterior cingulate cortex is sensitive to a dopaminergic agonist, such as apomorphine, is not unexpected. The marked metabolic activation in the frontal cortex and sensory motor cortex, particularly in the

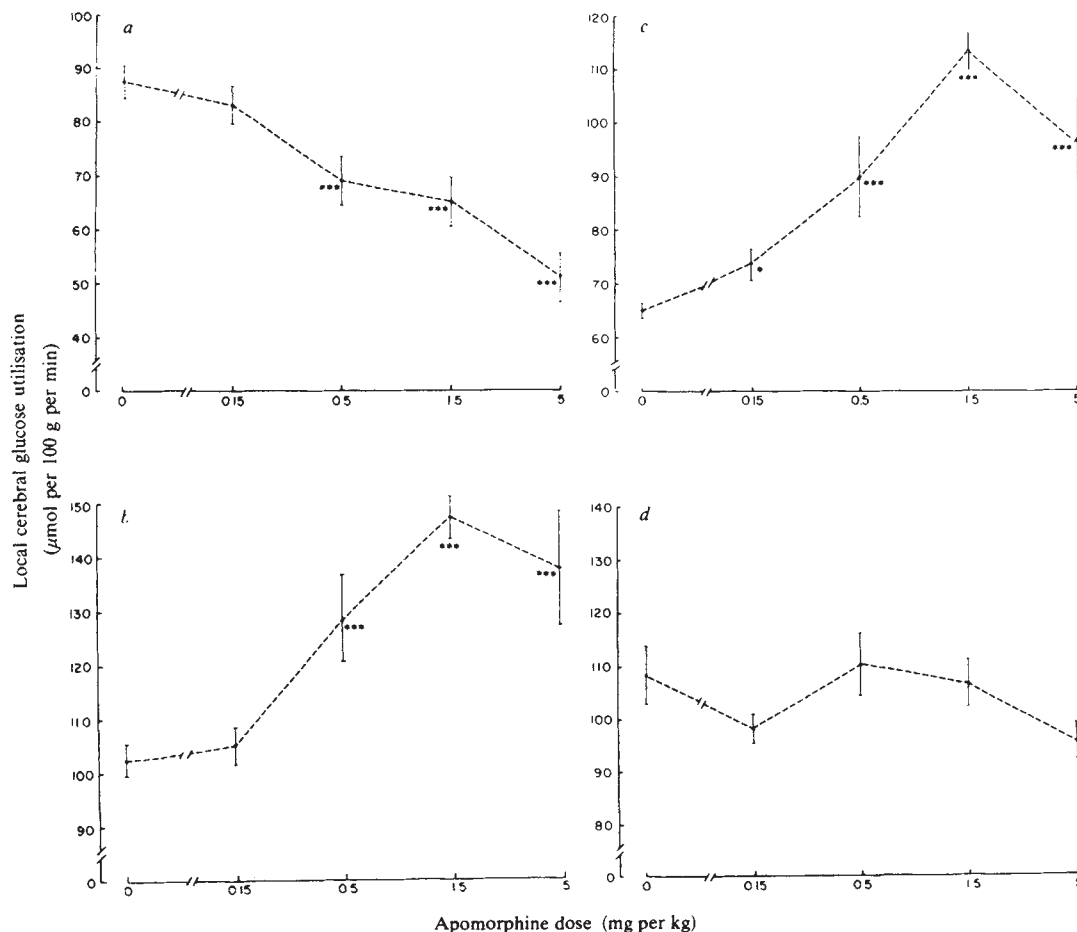


Fig. 2 Log dose-response curve for the rate of glucose utilisation in various cortical areas after intravenous injection of apomorphine. *a*, Anterior cingulate cortex; *b*, sensory motor cortex layer IV; *c*, sensory motor cortex layer VI; *d*, posterior parietal cortex. Data are from 25 animals and are presented as mean $\pm$ s.e.m.

* $P < 0.05$. *** $P < 0.001$.

deeper laminae, after administration of apomorphine, is of considerably greater interest. It is noteworthy that it is in layer VI and the deepest portion of layer V that the dopaminergic terminals⁴ and dopamine-sensitive cells⁹ have been identified in the rostral area of the medial aspect of the frontal cortex. There is little evidence, however, of a similar dopaminergic system in layer VI of the dorsolateral frontal cortex and the sensory motor cortex. The regions of cortex in which glucose utilisation increased after administration of apomorphine correspond closely with those areas to which the ventral nucleus of the thalamus projects^{10,11}. Glucose utilisation in this thalamic nucleus was increased after administration of apomorphine, and the metabolic activation observed in the dorsolateral frontal and sensory motor cortices may reflect, at least in part, increased activity of non-dopaminergic thalamocortical systems.

The interpretation of the results of any neuropharmacological investigation depends on the specificity of action of the agents used. In the study reported here, the sensitivity to apomorphine of glucose utilisation in areas such as layer VI of the sensory motor cortex, and the prevention of the apomorphine-induced alterations in glucose utilisation in all cortical areas by low doses of the dopamine-antagonist, haloperidol, suggest a key involvement of dopaminergic receptors in the responses observed. Our finding that the cortical involvement in the action of apomorphine extends beyond the known confines of the mesocortical dopaminergic system suggests a need for reappraisal of the mechanisms and foci of action underlying the behavioural effects of antipsychotic and other drugs, which are used to manipulate dopaminergic systems in the CNS.

J.McC. was supported by a Fogarty International post-doctoral fellowship.

Received 2 July; accepted 13 September, 1979.

1. Thierry, A. M., Blanc, G., Sobel, A., Stinus, L. & Glowinski, J. *Science* **182**, 499–501 (1973).
2. Lindvall, O., Björklund, A., Moore, R. Y. & Stenevi, U. *Brain Res.* **81**, 325–331 (1974).
3. Lindvall, O., Björklund, A. & Divac, I. *Adv. biochem. Psychopharmac.* **16**, 39–46 (1977).
4. Hökfelt, T., Fuxe, K., Johansson, O. & Ljundahl, A. *Eur. J. Pharmac.* **25**, 108–112 (1974).
5. Tassin, J. P. *et al. Brain Res.* **154**, 241–251 (1978).
6. Trabuchi, M., Govoni, S., Tonon, G. C. & Spano, P. F. *J. Pharm. Pharmac.* **28**, 244–245 (1976).
7. Sokoloff, L. *et al. J. Neurochem.* **28**, 897–916 (1977).
8. Sokoloff, L. *J. Neurochem.* **29**, 13–26 (1977).
9. Bunney, B. S. & Aghajanian, J. K. *Life Sci.* **19**, 1383–1392 (1976).
10. Jacobson, S. & Trojanowski, J. Q. *Brain Res.* **85**, 385–401 (1975).
11. Krettek, J. E. & Price, J. L. *J. comp. Neurol.* **171**, 157–192 (1977).

Growth and differentiation of aggregating fetal brain cells in a serum-free defined medium

Paul Honegger, Dominique Lenoir & Pierric Favrod

Institut de Physiologie, Faculté de Médecine, Université de Lausanne, Switzerland

Aggregating cultures of mechanically dissociated fetal brain cells provide an excellent system for neurobiological studies of cellular growth and differentiation^{1–5}, but, in common with almost all culture systems, they have the disadvantage that crude serum is required in the medium. Although several cell lines have either been adapted to serum-free conditions^{6–12} or grown normally in serum-free media supplemented with hormones, trace elements and defined serum components^{12–16}, this approach has never been applied to differentiating primary cells of the central nervous system. We now describe the successful cultivation of aggregating fetal rat brain cells in a chemically defined, serum-free medium.



Fig. 1 Electron micrograph of a 35-d aggregate grown in S^- medium, showing a myelinated axon and a synaptic contact. The tissue was fixed in paraformaldehyde–glutaraldehyde, postfixed in osmium tetroxide and embedded in Epon. Sections were stained with uranyl acetate and lead citrate ($\times 42,000$).

Equal numbers of mechanically dissociated fetal (15–16 d gestation) rat brain cells^{1,2} were inoculated either in Dulbecco–Vogt modified Eagle's medium (DMEM) containing fetal calf serum, referred to as S^+ medium, or in serum-free DMEM supplemented with insulin ($5 \mu\text{g ml}^{-1}$), 20 nM hydrocortisone, 0.3 nM triiodothyronine, transferrin $1 \mu\text{g ml}^{-1}$ and trace elements (see Table 1 legend), referred to as S^- medium. In either case the dissociated cells re-aggregated readily to give a uniform population of aggregates. Throughout the culture period, aggregates in S^- medium remained somewhat smaller, but more numerous (two or threefold) than those in S^+ medium.

Cell proliferation was examined by measuring both the total DNA content and the incorporation of ^3H -thymidine into a trichloroacetic acid (TCA)-precipitable macromolecular fraction (Table 1). Cultures grown in S^- medium (S^- cultures) had a greater DNA content and a smaller protein/DNA ratio than cultures grown in S^+ medium (S^+ cultures). In addition, cell counts of thionine-stained semi-thin sections of aggregates revealed almost twice as many cells per unit area in S^- than did S^+ cultures. Both observations suggest slower cell growth (for example, protein acquisition) in S^- cultures. Incorporation of ^3H -thymidine (representing DNA synthesis) was demonstrated in both S^+ and S^- cultures (Table 1). During the first 10 d of culture, S^- cultures showed a higher rate of DNA synthesis, with maximum activity after about 5 d. After 10 d, DNA synthesis diminished more rapidly in S^- than in S^+ cultures and by day 20 it was only about one-third of that of S^+ cultures. Lack of either insulin or transferrin in S^- medium resulted in a reduction of both total DNA and ^3H -thymidine incorporation at day 5, whereas no difference was observed in the initial rate of ^3H -thymidine uptake between cultures grown either in the presence

or absence of insulin. These results suggest that both insulin and transferrin stimulate cell proliferation in S^- cultures, and this agrees with results of investigations with established cell cultures grown in serum-free conditions^{12,14,16}. Lack of either hydrocortisone or triiodothyronine, two other hormones that stimulate the growth of some cell lines¹⁴, had no apparent effect on DNA synthesis.

The morphological differentiation of the aggregates was followed by electron microscopy. Compared with S^+ cultures, S^- cultures underwent generally slower maturation of neuronal processes and delayed myelin synthesis. In S^- cultures no synaptic profiles were found after 4 d, but they were frequent after 8 d; the first myelinated axons appeared at about day 30. After 35 d numerous synaptic contacts and many myelinated fibres were observed (Fig. 1). The compaction of myelin sheaths often appeared incomplete.

The biochemical differentiation of the cultures was studied at regular intervals by measuring the specific activities of the following neurotransmitter metabolising enzymes: choline acetyltransferase (CAT, EC 2.3.1.6), acetylcholinesterase (ACE, EC 3.1.1.7), glutamate decarboxylase (GAD, EC 4.1.1.15), aromatic L-amino acid decarboxylase (AAD, EC 4.1.1.26) and monoamine oxidase (MAO, EC 1.4.3.4). Table 2 shows that in S^- cultures, the specific activities of all enzymes (and the protein content) increased considerably during the first month. In contrast to S^+ cultures, the phase of rapid increase in enzyme specific activities occurred later and no plateau was reached within 4 weeks (with the exception of MAO). Compared with S^+ cultures, aggregates grown for 33 d in S^- had higher specific activities of GAD (192%), AAD (148%) and ACE (168%), but lower CAT (40%) and MAO (51%). Lack of insulin in S^- medium resulted in a significant reduction of both GAD activity and protein content, the reduction of GAD activity being more pronounced at early developmental stages (57% reduction at day 11 compared with 15% reduction

at day 33). Lack of transferrin in S^- caused a progressive decrease in protein content and the specific activities of all enzymes except MAO. On the other hand, lack of hydrocortisone or triiodothyronine had no apparent effect on the enzyme activities. In view of the relatively high GAD activity in S^- cultures and because the possible neuronal localisation of this enzyme required verification^{20,21}, we examined the formation of labelled γ -aminobutyric acid (GABA) from L-[U-¹⁴C]glutamate in aggregates (Fig. 2). Compared with S^+ cultures, S^- cultures took up glutamate at a higher rate (Fig. 2a) and had an almost two-fold greater net synthesis of GABA (Fig. 2b), the latter finding agreeing with the differences in GAD activity measured in homogenates (Table 2). The formation of GABA from labelled glutamate was measured in the presence of the carbonyl-trapping agent aminooxyacetic acid (AOAA). After 30 min of incubation in the presence of 13 μ M AOAA, synthesis in both S^+ and S^- aggregates of ¹⁴C-GABA was less than 5% of that in control cultures. This contrasts with studies of astrocyte cultures²¹, in which 13 μ M AOAA did not inhibit GABA formation. Furthermore, both GAD activity and rate of GABA synthesis were higher in aggregates than in astrocytes²¹ by at least an order of magnitude. Thus the GAD activity measured in aggregates probably represents the neuronal enzyme activity.

The accumulation of glutamine formed from labelled glutamate was considerably lower in S^- than in S^+ cultures (Fig. 2c). Glutamine synthetase (EC 6.3.1.2), the enzyme involved in the amidation of glutamate, has been shown to be localised in glial cells²², suggesting that S^- cultures contained relatively fewer glial cells than S^+ cultures. This is corroborated by the relatively low specific activity of MAO in S^- cultures. (Several observations suggest that the MAO activity of neuronal cells in culture is considerably lower than that of glia—ref. 23 and P.H., unpublished.) Thus, a proportionately higher number of neurones in S^- aggregates could fully account for the relatively

Table 1 DNA synthesis in aggregating cultures of fetal rat brain cells

Condition	Days in culture	TCA precipitable radioactivity ($10^{-6} \times$ d.p.m. per flask)	Total DNA (μ g per flask)	Total protein (mg per flask)	Protein/DNA
S^+	2	1.2	126	3.0	24
	5	3.6	155	4.0	26
	10	3.2	120	6.1	50
S^- , complete	2	3.9	316	4.0	13
	5	12.3	383	4.9	13
	10	6.0	270	7.0	26
S^- , no insulin	5	4.0	265	3.4	13
S^- , no transferrin	5	2.2	293	3.9	13
S^- , no hydrocortisone	5	11.1	356	4.6	13
S^- , no triiodothyronine	5	12.1	360	4.8	13

Brains of fetal (15–16 d gestation) Wistar rats (Madörin, Füllinsdorf) were dissected and dissociated mechanically as before^{1,2}. The cells were washed three times with Puck's D₁ solution by trituration and centrifugation (3,700g_{av} per min), and resuspended in serum-free Dulbecco–Vogt modification of Eagle's medium (DMEM, high glucose, no pyruvate, GIBCO no. 320–1965, supplemented with: vitamin B₁₂ 1.36 μ g ml⁻¹, Sigma; biotin 0.007 μ g ml⁻¹, Sigma; DL- α -tocopherol 10 μ g ml⁻¹, Sigma; retinol 5 μ g ml⁻¹, Fluka; lipoic acid 0.2 μ g ml⁻¹, Sigma; linoleic acid 0.1 μ g ml⁻¹, Sigma; penicillin 50 U ml⁻¹, ICN; and streptomycin sulphate 50 μ g ml⁻¹, ICN). This single cell suspension of 3×10^7 viable cells per ml was divided into two equal portions and then diluted with 2 volumes of either S^+ medium (DMEM containing 15% (v/v) fetal calf serum (Seromed) or S^- medium (DMEM without serum). The complete serum-free medium (S^-) contained the following additional supplements: crystalline bovine insulin (5 μ g ml⁻¹, Sigma) hydrocortisone-21-phosphate (20 nM, Sigma), 3,3',5-triiodo-L-thyronine (0.3 nM, Sigma), human transferrin (1 μ g ml⁻¹, Sigma) and various trace elements as listed by Hutchings and Sato¹⁵. Samples (3.5 ml) of the cell suspensions were placed into 25-ml De Long flasks (Bellco) and incubated at 37 °C in an atmosphere of 10% CO₂/90% humidified air, under constant rotation at 70 r.p.m. (Infors gyratory shaker). After 2 d, the cultures were transferred to 50-ml De Long flasks (Bellco) and 5-ml samples of new media were added. Media were changed (exchange of 5 ml medium) every 3 d. Within the first week of culturing, speed of rotation was increased gradually to 80 r.p.m. DNA synthesis was determined by measuring incorporation of ³H-thymidine into a TCA-precipitable macromolecular fraction¹⁷. Cultures were incubated for 22 h in normal conditions with [Me-³H]thymidine (Amersham, 46 Ci mmol⁻¹ specific activity) to give a final concentration of 25 nM (1.2 Ci ml⁻¹). Controls were incubated for 22 h at 4 °C. Aggregates were then washed three times with solution D₁, homogenised in 0.6 ml of 0.05% (v/v) Triton X-100 using a glass-Teflon homogeniser and sonicated briefly (Branson model B-12, 3 $\times$ 3 s at 10 W). Samples (25–100 μ l) of the homogenate were mixed with 1.5 ml of cold 10% (w/v) TCA, kept at 0 °C for 15 min and then collected by suction onto GF/A glass fibre filter disks. The filters were washed three times with 5-ml samples of 10% TCA, transferred to glass counting vials and incubated for 12 h in 0.5 ml NCS tissue solubiliser (Amersham). After neutralisation of the digest with glacial acetic acid, 10 ml scintillation cocktail (toluene, containing 6 g l⁻¹ of 2,5-diphenyloxazole (PPO) and 75 mg ml⁻¹ of 1,4-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP)) was added for liquid scintillation counting. Portions of homogenates were assayed for protein by a modification of the method of Lowry *et al.*¹⁸ and for DNA by a modification of the method of Kissane and Robins¹⁹. The values given are the mean of at least two individual cultures (deviation < 10%). The average radioactivity found in controls (2,600 d.p.m. per mg protein) was subtracted.

Table 2 Development of specific activities of neurotransmitter metabolising enzymes in aggregating cultures of fetal rat brain cells

Condition	Days in culture	Specific enzyme activity (pmol per min per mg protein)					Total protein (mg per flask)
		CAT	ACE	GAD	AAD	MAO	
S ⁺	11	50	16,100	335	61	320	6.0
	22	225	39,300	776	67	890	8.8
	33	305	45,200	1,137	110	1,325	9.2
S ⁻ , complete	11	11	8,700	376	64	203	5.9
	22	48	26,600	946	91	428	6.8
	33	122	76,100	2,187	163	680	8.8
S ⁻ , no insulin	11	16	5,800	161	42	112	3.4
	22	53	24,600	485	66	381	3.4
	33	148	54,500	1,858	185	666	5.0
S ⁻ , no transferrin	11	7	6,100	307	42	140	5.5
	22	6	13,900	230	40	390	3.9
	33	3	16,100	209	20	670	3.2
S ⁻ , no hydrocortisone	11	8	8,300	330	50	212	5.7
	22	46	24,600	981	76	376	7.1
	33	119	73,600	1,968	131	761	8.5
S ⁻ , no triiodothyronine	11	10	8,200	382	64	212	6.4
	22	41	25,300	869	93	392	6.7
	33	110	69,500	2,050	143	675	8.8

Fetal rat brain cell aggregates were prepared and cultivated in DMEM containing 15% (v/v) fetal calf serum (S⁺) or in serum-free DMEM (S⁻), as described in Table 1 legend. The methods used for homogenate preparation and enzyme assays have been described before¹. Protein was determined by a modification of the method of Lowry *et al.*¹⁸, using bovine serum albumin as a standard. The values given represent the mean of at least five individual culture flasks (s.e.m. < 5%).

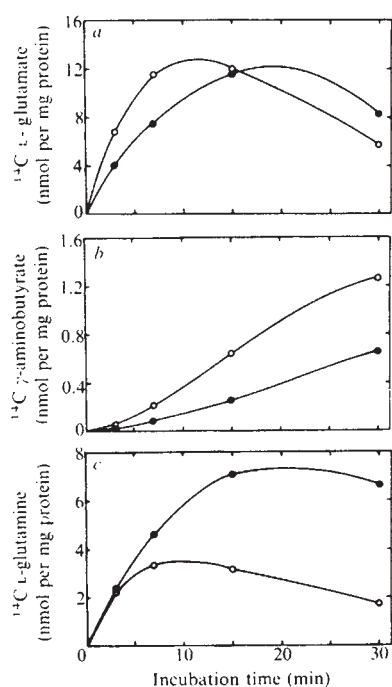


Fig. 2 Aggregates cultured for 30 d in either S⁺ medium (●) or in S⁻ medium (○) were preincubated for 30 min in serum-free DMEM deficient in L-glutamine and subsequently incubated in the presence of 100 μM L-[U-¹⁴C]glutamate (Amersham, 1.4 mCi mmol⁻¹ final specific activity). The commercially available radioactive material was purified before use by TLC on cellulose plates. The solvent system was 1-butanol/glacial acetic acid/water: 12/3/5 (v/v). After various times, aggregates were analysed for their content of L-¹⁴C-glutamate (a), L-¹⁴C-GABA (b) and L-¹⁴C-glutamine (c). The labelled compounds were extracted from homogenates of the washed aggregates and then quantitatively separated on cellulose plates by using the following two-dimensional separation technique: high voltage electrophoresis (buffer: 0.125 M pyridine, 1.05 M acetic acid, pH 3.8), followed by TLC (solvent system as above). Further technical details are given in ref. 5. Quantities of products were calculated on the assumption that the specific activity of the precursor in the tissue was equal to that in the medium. Aliquots of the homogenates were assayed for protein by a modification of the method of Lowry *et al.*¹⁸ (s.e.m. < 5%).

high GAD activity observed in these cultures. If this is the case, the relatively low specific activity of CAT, another neuronal marker enzyme, may be explained if, in S⁻ cultures, (1) there is a smaller proportion of cholinergic neurones; (2) there is a greater retardation in the development of cholinergic neurones; or (3) CAT is more retarded in its development than GAD. Although the present results do not enable us to distinguish between these possibilities, there is some circumstantial evidence in favour of points (2) and/or (3), that is, compared with S⁻ cultures of cells from the mesencephalon–diencephalon–rhombencephalon region, S⁻ cultures of telencephalic cells (which are presumably at an earlier stage of development) show a much higher rate of DNA synthesis, a much more pronounced delay of CAT and some delay of GAD maturation (data not shown).

In conclusion, our morphological and biochemical data demonstrate that mechanically dissociated fetal rat brain cells re-aggregate, grow and differentiate in a chemically defined, serum-free medium. Such cultures show some retardation in cellular growth and differentiation compared with their counterparts grown in the presence of 15% fetal calf serum. Although more work is needed to define their developmental characteristics further, serum-free re-aggregating brain cell cultures will be valuable for the study of nutritional and hormonal influences on brain development.

We thank Professor M. Dolivo for support and encouragement, Ms C. de Weck and Mr C. Verdan for technical assistance, and Dr G. Fagg for help with the manuscript. This work was supported by Swiss NSF grant 3.117.77.

Received 14 March; accepted 25 September 1979.

- Honegger, P. & Richelson, E. *Brain Res.* **109**, 335–354 (1976).
- Honegger, P. & Richelson, E. *Brain Res.* **133**, 329–339 (1977).
- Matthieu, J.-M., Honegger, P., Trapp, B. D., Cohen, S. R. & Webster, H. DeF. *Neuroscience* **3**, 565–572 (1978).
- Trapp, B. D., Honegger, P., Richelson, E. & Webster, H. DeF. *Brain Res.* **160**, 117–130 (1979).
- Honegger, P. & Richelson, E. *Brain Res.* **162**, 89–101 (1979).
- Waymouth, C. J. *natn. Cancer Inst.* **22**, 1003–1017 (1959).
- Katsuta, H. & Takaoka, T. *Meth. Cell Biol.* **6**, 1–42 (1973).
- Katsuta, H. & Takaoka, T. *Meth. Cell Biol.* **14**, 145–158 (1976).
- Donta, S. T. *Meth. Cell Biol.* **9**, 123–137 (1975).
- Higuchi, K. *Meth. Cell Biol.* **14**, 131–143 (1976).
- Ariano, T. *Neurochem. Res.* **2**, 342 (1977).
- Ham, R. G. & McKeehan, W. L. *In Vitro* **14**, 11–22 (1978).
- Izumi, H. & Sato, G. H. *Nature* **259**, 132–134 (1976).
- Hayashi, I., Larner, J. & Sato, G. H. *In Vitro* **14**, 23–30 (1978).
- Hutchings, S. E. & Sato, G. H. *Proc. natn. Acad. Sci. U.S.A.* **75**, 901–904 (1978).
- Bottenstein, J. E. & Sato, G. H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 514–517 (1979).
- Schrier, B. K. & Wilson, S. H. *Meth. Cell Biol.* **13**, 105–120 (1976).

18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).
19. Kissane, J. M. & Robins, E. *J. biol. Chem.* **233**, 184–188 (1958).
20. Wu, J.-Y. *et al.* *J. Neurochem.* **30**, 849–857 (1978).
21. Wu, P. H., Durden, D. A. & Hertz, L. *J. Neurochem.* **32**, 379–390 (1979).
22. Martinez-Hernandez, A., Bell, K. P. & Norenberg, M. D. *Science* **195**, 1356–1358 (1977).
23. Richelson, E. in *Handbook of Psychopharmacology* 1 (eds Iversen, L. L., Iversen, S. D. & Snyder, S. H.) 101–135 (Plenum, New York, 1975).

Androgen receptors exist throughout the 'critical period' of brain sexual differentiation

Christine C. Vito, Steven J. Wieland & Thomas O. Fox

Department of Neuropathology, Harvard Medical School, and Department of Neuroscience, Children's Hospital Medical Center, 300 Longwood Avenue, Boston, Massachusetts 02115

Several studies suggest direct roles for androgens and oestrogens in the development of sexually dimorphic characteristics of mouse and rat brain¹. To elucidate the biochemical mechanisms for such effects, investigators have asked which putative steroid receptors are present in the hypothalamus throughout the critical period of sexual differentiation. *A priori*, potential receptors would include high-affinity proteins with selectivities for active androgens, oestrogens and their metabolites. Two major classes of steroid that might be active as agonists are the androgens *per se*, including testosterone and dihydrotestosterone (DHT)², and the oestrogens, which are themselves androgen metabolites³. Normally, in sexual differentiation, a proper balance of both androgens and oestrogens may be necessary⁴. Indeed, receptors for each of these ligands do exist in the hypothalamus of neonatal and prepubertal mice^{4–7}. Although the perinatal oestrogen receptor and its function have been extensively studied^{7,8}, the existence of perinatal androgen receptors has not been as clearly demonstrated to permit a similar acceptance⁹. In this report, we establish the existence of adult-like androgen receptors in embryonic and neonatal mouse and rat hypothalami by qualitative biochemical and genetic analyses. This is achieved by DNA-cellulose affinity chromatography and velocity sedimentation, and by analysis of the androgen-resistant mutant, testicular feminisation. The presence of sex hormone receptors in perinatal brain is discussed in the context of behavioural responses which are differentiated during the critical period of brain sexual development.

We used DNA-cellulose affinity chromatography to characterise the androgen-binding proteins in perinatal hypothalamus as it fractionates low levels of androgen receptor in prepubertal brain^{10,11} and permits qualitative analysis of the DNA-adhering material^{7,8}. Figure 1 shows a representative elution profile of prepubertal hypothalamic androgen receptor (triangles), and also illustrates (circles) a ³H-DHT-binding activity from neonatal hypothalamus which similarly adheres to DNA-cellulose and elutes in the same manner with a linear concentration gradient of NaCl. The androgen-binding activities in both neonatal and prepubertal cytosols exhibit elution maxima in the

140–150-mM NaCl range of the gradient. Therefore, by the criteria of DNA adherence, the androgen-binding activity in perinatal mouse hypothalamus is qualitatively similar to the putative androgen receptor present in older animals.

To determine its macromolecular integrity, the material which elutes from DNA-cellulose was analysed by velocity sedimentation (Fig. 2). As indicated by the lack of radioactivity at or near the top of sucrose gradients following centrifugation, the androgen is indeed bound to macromolecular components of cytosols from prepubertal (21 days after birth; Fig. 2a), neonatal (3–4 days after birth; Fig. 2b, closed circles) and embryonic (3 days before birth; Fig. 2b, open circles) hypothalami. The androgen-binding activities from both neonatal and embryonic hypothalami (Fig. 2b) behave similarly to that from prepubertal hypothalamus (Fig. 2a) in that all sediment as 4S macromolecules. The sedimentation profiles shown in Fig. 2a and b are representative of data obtained at all hormone concentrations tested.

Using both DNA-cellulose chromatography and velocity sedimentation, we have assessed the saturability of the androgen-binding activities in embryonic, neonatal and prepubertal tissues. Androgen-binding activities are $92 \pm 3\%$ (s.e.m. $n = 15$) saturated at hormone concentrations below 10 nM; this is observed with both DHT and testosterone in both hypothalamic and kidney cytosols, and agrees with previous observations that putative androgen receptor from prepubertal animals saturates at hormone concentrations between 4 and 8 nM (refs 4, 7).

In mature animals with the androgen-resistant genetic syndrome, testicular feminisation (*Tfm*), the levels of androgen receptor are lower in both hypothalamus^{4,10–12} and kidney^{7,13–15} than in wild-type animals. As the wild-type neonatal androgen-binding activity which adheres to DNA-cellulose seems to be similar to that of older animals (Figs 1, 2), we would expect this activity in neonatal *Tfm/Y* tissues to be similarly affected if the mutation is expressed at an early age. Indeed, the androgen binding capacity of neonatal *Tfm/Y* cytosols (hypothalamus and kidney) is approximately 15% that of sibling (male and female) cytosols (Table 1); a similar observation has been made in submandibular gland and kidney cytosols using sedimentation analysis¹⁶. In contrast, the concentration of oestrogen receptor in mouse *Tfm/Y* hypothalamus is similar to that in sibling hypothalamus at the neonatal ages tested (data not shown), as was shown for older animals^{4,10}.

Thus, our data suggest that both embryonic and neonatal hypothalamus contain an androgen-binding activity which is qualitatively similar to the putative androgen receptor in the hypothalamus of older animals. However, the level of neonatal binding is lower than prepubertal binding (Figs 1, 2).

Using the qualitative DNA-cellulose and velocity sedimentation patterns presented above, we have also quantified the putative androgen receptor content of the developing mouse hypothalamus. These data are summarised in Fig. 3. The concentration of androgen receptor detected in mouse hypothalamus between embryonic and prepubertal ages increases approximately sevenfold. In contrast, between 3 days before and 3 days after birth, only a twofold increase in the concentration of androgen receptor is detected. These data augment earlier quantitative measurements which also indicate an increase between late postnatal and prepubertal ages^{6,17}. Thus, the phase of most rapid appearance of androgen receptor seems to coincide with the late phase of the critical period of brain sexual differentiation. This ontogeny differs significantly from that of oestrogen receptors in mouse hypothalamus, as the overall increase in the concentration of oestrogen receptor from embryonic to prepubertal ages is only twofold^{7,8}. It is possible that technical or biological factors which differentially affect the detectability of androgen receptors cause this apparent difference. However, in spite of this qualification concerning the levels of androgen receptors, the significance of our report is that it establishes their presence in the hypothalamus throughout perinatal development.

Table 1 Androgen receptors in neonatal *Tfm/Y* and sibling mice

		Testosterone bound (mol $\times 10^{18}$ per mg tissue)*	
Age (d)	Genotype	Hypothalamic-preoptic area	Kidney
5	♀	6.7	42.0
	<i>Tfm/Y</i>	1.1	3.9
7	♀	7.0	50.0
	♂	7.5	64.0
	<i>Tfm/Y</i>	0.9	5.5

* Quantification was by DNA-cellulose affinity chromatography, using a protocol similar to that described in Fig. 1 legend.

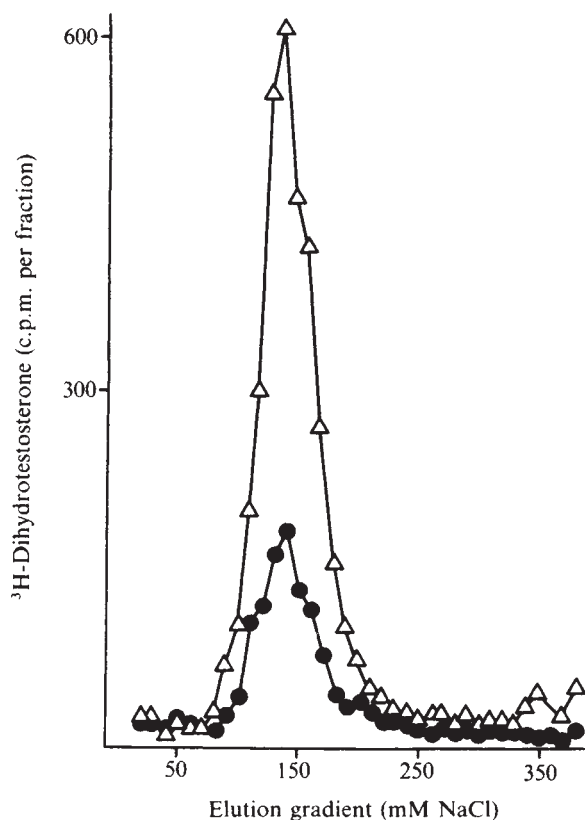


Fig. 1 DNA-cellulose affinity chromatography of androgen receptor from cytosols of neonatal and prepubertal mouse hypothalamus. C57BL/6J mice were obtained from the timed-pregnancy colony in our department. To prepare cytosol extracts, hypothalamic-preoptic area blocks from either 4- and 5-d-old mice (●) or 20- and 21-d-old mice (△) were homogenised at 2 °C, using a Kontes Teflon/glass homogeniser, in buffer containing: 10 mM Tris-HCl (pH 8.1, 21 °C), 1 mM mercaptoethanol, 1 mM Na₃EDTA, 10% glycerol and 50 mM NaCl. After the homogenate had been centrifuged at 140,000 g for 60 min, the resulting cytosol was collected and incubated with 10 nM [1,2,4,5,6,7-³H(N)]5 α -dihydrotestosterone (DHT; 152 Ci mmol⁻¹, NEN). Before use, we determined the purity of radioactively labelled steroid by HPLC (high-performance liquid chromatography: Waters Associates Inc.) or TLC (thin-layer chromatography); it was necessary to use purified ³H-DHT which was obtained by HPLC. Following a 60-min incubation with ³H-DHT, the cytosol preparations (each containing an equivalent of 970 mg tissue) were loaded on columns containing 5 ml DNA-cellulose which had been equilibrated with buffer containing 50 mM NaCl. The columns were then washed with approximately 60 ml of buffer during a 12-h period. The ³H-DHT-bound content of the column was eluted with a 60-ml linear gradient of NaCl, ranging from 50 to 500 mM. For each column, fractions were collected and the NaCl concentrations were determined using a Radiometer conductivity meter. Radioactivity was measured after the addition of 5.0 ml of Omnifluor (NEN)-toluene scintillation fluid.

In rat hypothalami at embryonic, neonatal and prepubertal ages, we have also detected androgen-binding activities which adhere to DNA-cellulose and elute sharply at a NaCl concentration of approximately 140 mM. These elution characteristics are similar to those shown in Fig. 1. Saturable androgen-binding capacities of rat hypothalamic cytosols are, respectively, 5.8 ± 1.4 (2–4 days before birth; $n = 3$), 7.7 ± 0.3 (4 days after birth; $n = 2$) and 37.8 ± 8.4 (21–35 days after birth; $n = 3$) mol $\times 10^{18}$ per mg tissue ($\pm$ s.e.m.). In keeping with the mutant mouse results, the androgen-binding activity in the hypothalamus of the androgen-resistant rat¹⁸ is approximately 20% that of the wild type.

Perinatal administration of DHT masculinises some features of both sexual behaviour in rats¹⁹ and guinea pigs²⁰, and social play in monkeys²¹. The masculine behavioural potential of both aggressive behaviour in female mice^{22,23} and sexual behaviour in female rats²⁴ is influenced by the *in utero* proximity to males, presumably by androgens from testicular secretions. These *in utero* masculinising effects on sexual behaviour are blocked by flutamide²⁴, a presumed antagonist of direct androgen action. However, masculinisation can occur fully^{25,26}, or in part²⁷, after postnatal administration of presumed inhibitors of androgen conversion to oestrogens.

In addition to masculinisation, perinatal administration of androgens or oestrogens can suppress some features of female sexual behaviour in mice and rats²⁸. Moreover, androgens including DHT can defeminise some features of sexual behaviour in hamsters²⁹. On the other hand, prenatal exposure to flutamide³⁰ and 1,4,6-androstatriene-3,17-dione (ATD)³¹, which presumably block direct androgen action and oestrogen formation, respectively, enhance lordotic behaviour in female rats. As female sexual behaviour can be suppressed by both androgens and oestrogens and enhanced by agents which block their direct actions, both hormones may mediate these developmental processes. These experiments suggest that androgen receptors could be involved in some aspects of masculinisation and defeminisation of sexually dimorphic behaviours. The present findings provide one possible molecular mechanism

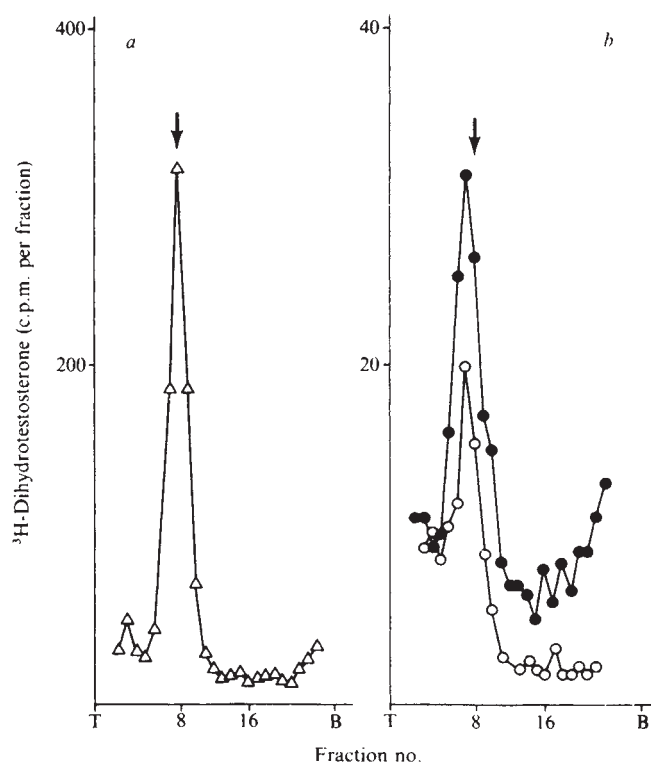


Fig. 2 Velocity sedimentation analysis of androgen receptor from embryonic, neonatal and prepubertal mouse hypothalamus. Hypothalamic blocks from C57BL/6J mice of determined embryonic, neonatal and prepubertal ages were used. Cytosols (containing an equivalent of 300 mg tissue) were prepared, incubated with 20 nM ³H-DHT and chromatographed with DNA-cellulose as described in Fig. 1 legend. The ³H-DHT-bound contents of each column were eluted with a single step of buffer containing 400 mM NaCl and then layered on a sucrose gradient (5 ml; 5% to 20%, w/v) containing the same buffer. Gradients were centrifuged for 20 h at 234,000g (average force) and contained the internal fluorescent marker dansyl-BSA (4.7S), the position of which is indicated by the arrow. *a*, Prepubertal cytosols (21 days after birth), Δ — Δ . *b*, Embryonic cytosols (3 days before birth), $\bigcirc$ — $\bigcirc$; neonatal cytosols (4 and 5 days after birth), $\bullet$ — $\bullet$.

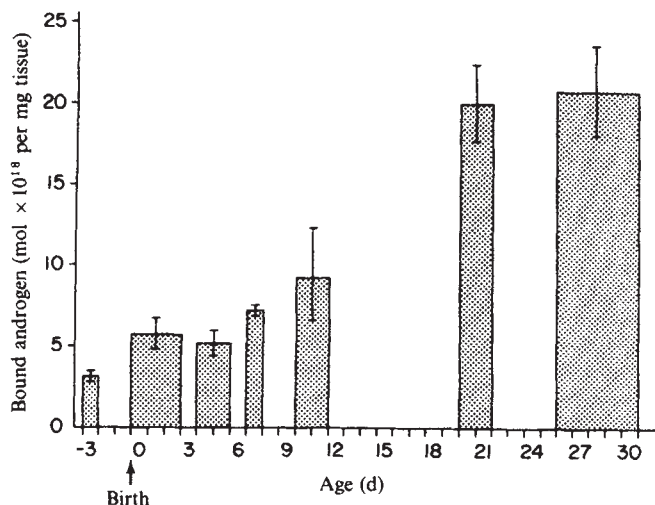


Fig. 3 Concentration of androgen receptors detected in the developing mouse hypothalamus. The horizontal axis represents time in days, with the distance between two markings representing one 24-h period. The vertical axis represents mol of bound androgen obtained by DNA-cellulose chromatography alone or in combination with velocity sedimentation. Velocity sedimentation analysis following DNA-cellulose chromatography was carried out at ages considered representative of embryonic (3 days before birth), neonatal (4–5 days post-birth) and prepubertal (21 days post-birth) development; the macromolecular material recovered following sedimentation was $95.6 \pm 3.9\%$ (s.e.m., $n = 8$) of the DNA-cellulose eluate applied to the sucrose gradient. The hormone-binding activities which are quantified as receptor activities are those which adhere to and eluate from DNA-cellulose in a specific manner. For example, in Fig. 1, the neonatal binding activity which elutes within the 75–225-mM region of the NaCl gradient is quantified by summing all those data points. This type of analysis serves as a qualitative control for the quantitative determinations reported above. Error bars indicate the s.e.m. The number of determinations (n) and the mean number of hypothalami per determination (HPOA) for the ages represented above are: 3 days before birth ($n = 2$, HPOA = 95); 0–2 days post-birth ($n = 2$, HPOA = 32); 4–5 days post-birth ($n = 6$, HPOA = 26); 7 days post-birth ($n = 2$, HPOA = 10); 10–11 days post-birth ($n = 3$, HPOA = 10); 20–21 days post-birth ($n = 6$, HPOA = 11); 25–30 days post-birth ($n = 2$, HPOA = 10). Our assignment system for embryonic ages has been described previously⁸. Records of vaginal plugs and births were used for all the age determinations illustrated above.

through which androgens may act as effectors of sexual differentiation.

We thank Sarah E. Bates and Kathie L. Olsen for their contributions to this work. Parts of the work were supported by a postdoctoral National Research Service Award (NICHD, to C.C.V.), a predoctoral National Research Service Award in Cell and Developmental Biology (grant 5 T32 GM 07226 to S.J.W.), a grant (HD 10818) and Research Career Development Award (NICHD to T.O.F.), and a grant from The National Foundation-March of Dimes. The work was carried out in the facilities of the Mental Retardation Research Center (NICHD) at the Children's Hospital Medical Center.

Received 16 April; accepted 7 September 1979.

1. Barracough, C. A. in *Advances in Reproductive Physiology* (ed. McLaren, A.) (Logos, London, 1968).
2. Farnsworth, W. E. & Brown, J. R. *J. Am. med. Ass.* **183**, 140–143 (1963).
3. Heard, R. D. H., Jellinek, P. H. & O'Donnell, V. J. *Endocrinology* **57**, 200–204 (1955).
4. Fox, T. O. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4303–4307 (1975).
5. Fox, T. O. *Nature* **258**, 441–444 (1975).
6. Attardi, B. & Ohno, S. *Endocrinology* **99**, 1279–1290 (1976).
7. Fox, T. O., Vito, C. C. & Wieland, S. J. *Am. Zool.* **18**, 525–537 (1978).
8. Vito, C. C. & Fox, T. O. *Science* **204**, 517–519 (1979).
9. Plapinger, L. & McEwen, B. S. in *Biological Determinants of Sexual Behavior* (ed. Hutchinson, J. B.) (Wiley, New York, 1978).
10. Wieland, S. J., Fox, T. O. & Savakis, C. *Brain Res.* **140**, 159–164 (1978).
11. Fox, T. O. *Brain Res.* **128**, 263–273 (1977).
12. Attardi, B., Geller, L. N. & Ohno, S. *Endocrinology* **98**, 864–874 (1976).

13. Attardi, B. & Ohno, S. *Cell* **2**, 205–212 (1974).
14. Bullock, L. P. & Bardin, C. W. *Endocrinology* **94**, 746–756 (1974).
15. Gehring, U. & Tomkins, G. M. *Cell* **3**, 59–64 (1974).
16. Verhoeven, G. & Wilson, J. D. *Endocrinology* **99**, 79–92 (1976).
17. Kato, J. *Ann. Biol. Anim. Biochem. Biophys.* **16**, 467–469 (1976).
18. Stanley, A. J., Gumbreck, L. G., Allison, J. E. & Easley, R. B. *Recent Prog. Horm. Res.* **29**, 43–64 (1973).
19. Hart, B. L. *Horm. Behav.* **8**, 193–200 (1977).
20. Goldfoot, D. A. & Van Der Werff Ten Bosch, J. J. *Horm. Behav.* **6**, 139–148 (1975).
21. Goy, R. W. in *Recent Advances in Primatology* Vol. 1 (eds Chivers, D. J. & Herbert, J.) (Academic, New York, 1978).
22. Gandelman, R., vom Saal, F. S. & Reinisch, J. M. *Nature* **266**, 722–724 (1977).
23. Vom Saal, F. S. & Bronson, F. H. *Biol. Reprod.* **19**, 842–853 (1978).
24. Clemens, L. G., Gladue, B. A. & Coniglio, L. P. *Horm. Behav.* **10**, 40–53 (1978).
25. Vreeburg, J. T. M., van der Vaart, P. D. M. & van der Schoot, P. J. *Endocr.* **74**, 375–382 (1977).
26. Davis, P. G., Chaptal, C. V. & McEwen, B. S. *Horm. Behav.* **12**, 12–19 (1979).
27. Booth, J. E. *J. Endocr.* **79**, 69–76 (1978).
28. Harris, G. W. *Endocrinology* **75**, 627–648 (1964).
29. Gerall, A. A., McMurray, M. M. & Farrell, A. J. *Endocr.* **67**, 439–445 (1975).
30. Gladue, B. A. & Clemens, L. G. *Endocrinology* **103**, 1702–1709 (1978).
31. Clemens, L. G. & Gladue, B. A. *Horm. Behav.* **11**, 190–201 (1978).

A region of the *Drosophila* genome necessary for CNS development

F. Jiménez & J. A. Campos-Ortega

Institut für Biologie III, Schänzlestr. 1, 7800 Freiburg i.Br., FRG

Mutations in genes involved in essential aspects of central nervous system development in *Drosophila melanogaster* are expected to be lethal. Thus, when searching for neurogenic mutants attention should be focused on embryonic lethal point mutants¹, for many of these might affect neural development. However, this approach can be very time consuming, for the location of neurogenic genes is unknown. A more convenient approach, which allows a faster screening of the genome, is to use relatively small chromosome deletions^{2,3} to determine whether the lack of a definite part of the genome affects neurogenesis. Once any region producing an interesting neural phenotype is found, it can be further analysed by the use of smaller deletions or point lethal mutants mapping within it, until the gene(s) responsible can be more precisely localised^{1,4}. We report here on a region of the *Drosophila* genome which has been found necessary for normal neurogenesis.

Using the technique designed by Zalokar and Erk⁵ for staining embryos as whole mounts, we have studied the effect on embryogenesis of deficiencies (a total of 52) covering ~1,000 bands distributed throughout all major chromosomes of *Drosophila*. We have found that some of them, even large ones, have no effect on the overall morphological pattern of the embryo, whereas others produce rather general morphogenetic defects. We intend to describe these defects in detail elsewhere. In addition to those deficiencies, however, we have found that some at the tip of the first chromosome exclusively alter the morphology of the embryonic nervous system in hemizygous animals, almost entirely eliminating neural tissue without modifying that of the remaining organs. Part of the chromosome region responsible for this abnormal phenotype is the *achaete-scute* system, which it has recently been suggested participates in neural development^{6,7}. We have examined the neural phenotype produced by terminal deficiencies of increasing size—*Df(1)sc⁸*, *Df(1)sc^H*, *Df(1)260-1*, *Df(1)y^{74k}*, *Df(1)svr* and *Df(1)Bld*—as well as some intercalary ones—*In(1)sc^{4L}sc^{9R}*, *Df(1)sc¹⁹*—with the following results (see Fig. 1). *Df(1)sc⁸*, the smallest terminal deficiency studied, does not seem to change the pattern of the nervous system observed in wild-type embryos. However, all remaining deletions eliminate increasing amounts of neural tissue, the extent of the lesion being correlated with the size of the chromosome deletion considered. The smallest intercalary deficiency studied (*In(1)sc^{4L}sc^{9R}*) only produces a main constriction in the middle of the ventral cord, whereas embryos hemizygous for the largest terminal deficiency

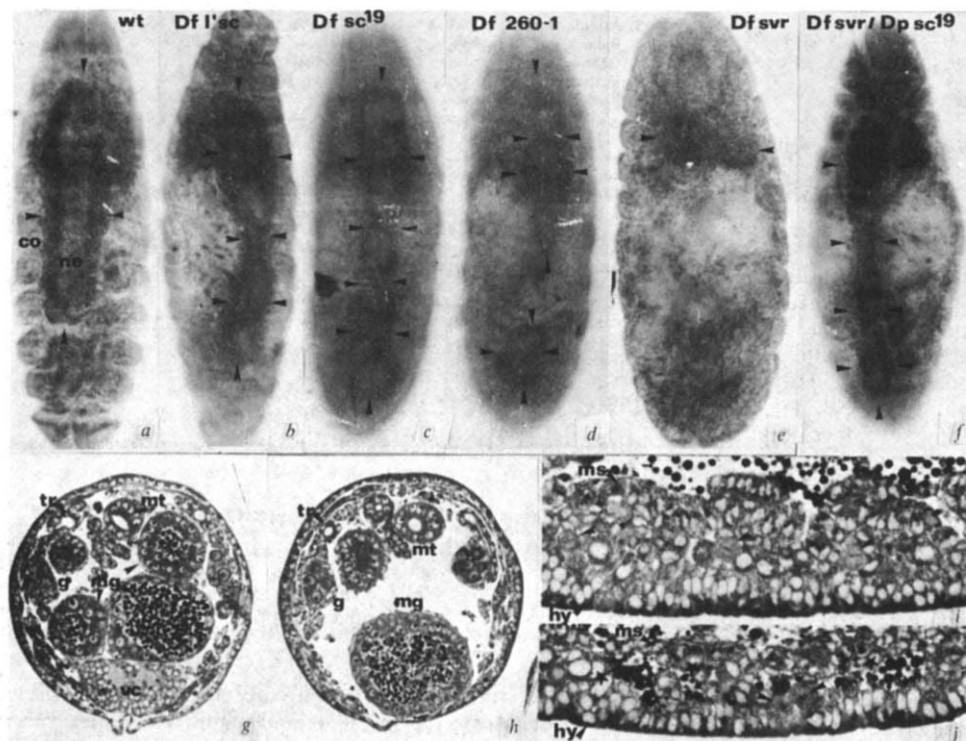
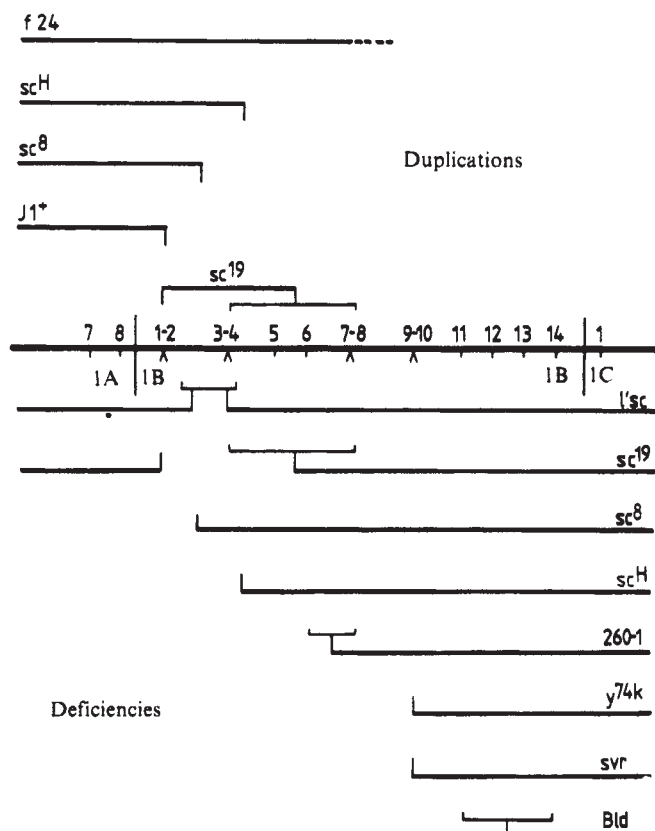


Fig. 1 Neural phenotype found in embryos hemizygous for deficiencies at the tip of the first chromosome. The experimental crosses were made with F_1 females, derived from an outcross of the deficiency-carrying stock to a wild-type (Oregon S) stock and wild-type males. The only exception was $Df(1)svr-Dp(1)sc^{19}$, in which $XX/Y; Dp(1)sc^{19}/CyO$ females were crossed with $Df(1)svr/y^2Y67g$ males. Embryos collected at 17 ± 1 (a-h) and 7 ± 0.5 (i, j) h after oviposition (25°C) were fixed either with alcohol/acetic acid/formaldehyde for fuchsin staining of whole mounts or with glutaraldehyde-osmium tetroxide for Durcupan embedding for light and electron microscopy⁵. **a** Shows a whole mount of a wild-type embryo. **b**, $Df(1)sc$, corresponding to $In(1)sc^{4L}sc^{9R}$ which includes the lethal of scute ($l'sc$)^{7,11}, produces a constriction in the abdominal ganglia in the ventral cord, which has not completely undergone the condensation normally occurring at this time⁸ (16–18 h of development). Constrictions and lack of condensation become more evident in $Df(1)sc^{19}$ (c) or in $Df(1)sc^H$ (not shown) embryos, both deficiencies producing a comparable phenotype. **d** Is an example of $Df(1)260-1$ embryos, showing a large gap between rostral and caudal parts of the ventral cord. **e** Illustrates the phenotype found in $Df(1)svr$ embryos, in which only remnants of the brain lobes are visible. **f** Shows the result of covering the left part

of the region of interest with the $Dp(1;2)sc^{19}$; the phenotype obtained is similar to that of the deficiency for the *achaete-scute* system $Df(1)sc^{19}$ shown in c. Photographs were taken focusing the subesophageal ganglion and the ventral cord so that the brain lobes are not clearly defined. The arrowheads mark the borders of the nervous system, co, cortex; ne, neuropile. **g, h**, Transverse sections at the level of the gonads through wild-type (**g**) and $Df(1)y^{74k}$ (**h**) embryos. **g**, gonads; **mg**, midgut; **mt**, malpighian tubules; **tr**, tracheae; **vc**, ventral cord. **i, j** Sagittal sections through the ventral region of wild-type (**i**) and $Df(1)svr$ (**j**) 7-h-old embryos. Dividing neuroblasts⁹ (arrowheads) in both instances and clusters of degenerating cells (asterisks) in the mutant are evident. **hy**, hypodermis; **ms**, mesoderm.

Fig. 2 Cytogenic location and extent of deficiencies and duplications used in the present work. In the centre of the figure, bands within subdivision 1B of the X chromosome are given according to Bridges' revised map¹². The lower half of the figure shows the deficiencies studied. Data of breakpoints are taken from refs 7, 10, 11 and 13. Some uncertainties exist concerning the location of deficiency breakpoints. For example, in $Df(1)l'sc$, corresponding to $In(1)sc^{4L}sc^{9R}$, genetic data^{7,14} demonstrate that $In(1)sc^9$ has a distal breakpoint to the right of that of $In(1)sc^4$; cytogenetically, however, the sequence is the inverse¹¹. The right breakpoint of $Df(1)sc^{19}$ has been found¹¹ at 1B4-7; genetically (ref. 7 and present work), it should be at the same location as that of $Df(1)sc^H$ —1B4—as the phenotype produced by both deficiencies is very similar. Finally, the breakpoint of $Df(1)Bld$ has been genetically and cytogenetically localised at 1B11-14. The upper half of the figure shows the extent of duplications according to refs 7 and 11 and to data from the present work.



cies (*Df(1)y^{74k}*, *Df(1)svr* and *Df(1)Bld*) do not show any organised ventral cord structure but only some cell clusters in the brain lobes (Fig. 1a–f).

We then studied serial sections of mutant embryos, and confirmed the previous observations. Note that in the mutants studied, all other embryonic structures that we are able to recognise on histological sections seem to be entirely normal at 17 ± 1 h of development. This can be seen, for example, in the sections shown in Fig. 1g and h, corresponding to comparable levels of wild-type and *Df(1)y^{74k}* embryos, where the only appreciable difference is the absence of the ventral cord in the mutant. However, a small but increasing number of dead cells is visible in the hypodermis of developing 10–14-h-old *Df(1)260-1*, *Df(1)svr* and *Df(1)Bld* embryos, although the hypodermis of 17 ± 1 -h-old embryos shows no defect in any of these mutants.

The nature of the lesion found at late stages of development was traced back by analysing younger mutant embryos—*Df(1)sc¹⁹*, *Df(1)260-1* and *Df(1)svr*. No abnormalities were detected at 4 h, but 7-h-old animals already displayed extensive cell death throughout both brain lobes and ventral cord anlagen (Fig. 1i, j), the extent being largely dependent on the deficiency considered, and the definitive phenotype being eventually attained at 13 h of development. As dividing neuroblasts seem to be present⁸ in the mutant, cell death must chiefly affect ganglion mother cells⁹ and/or differentiating neurones. From the above results, we suggest that the part of the genome responsible for this phenotype codes for functions necessary for the development of the nervous system. A more detailed analysis has been made feasible by covering part of the deleted region with corresponding pieces of the wild-type first chromosome translocated into other chromosomes. This analysis has demonstrated that the region to the right of *scute* also affects neural development (see Fig. 2). For example, by covering the left part of the region of interest with duplications, as in *Df(1)svr-Dp(1;2)sc¹⁹* (Fig. 1f) or *Df(1)svr-Dp(1;4)sc^H*, a phenotype roughly comparable to that of deletions of the *achaete-scute* system (for example, *Df(1)sc¹⁹*, (Fig. 1c) or *Df(1)sc^H*) is obtained. Thus, the analysis of deficiency–duplication combinations demonstrates that both the *achaete-scute* system and the region to its right might contain qualitatively similar signals.

The left border of the region under study is defined by *Dp(1;Y)l(1)J1⁺*; it is the largest terminal duplication of those we have studied which is unable to rescue the phenotype of either *Df(1)260-1* or *Df(1)svr*. The right border may be tentatively placed in the vicinity of the breakpoint of *Df(1)Bld* (see Fig. 2). This leaves us with a relatively large fraction of the *Drosophila* genome—almost all the subdivision 1B of the first chromosome—apparently coding for functions of related nature, as judged from the resulting phenotype. The problem of how specifically the whole region controls neural functions requires further investigation. Cell death in the developing hypodermis of some mutants, and the fact that *Df(1)svr* has been reported to be inviable in clones of imaginal epidermal cells whereas clones of *Df(1)260-1* cells are viable¹⁰, suggest that some gene functions included in the *Df(1)svr* might also be needed by tissues other than the nervous system. However, *Df(1)svr* is a large deletion, comprising at least 16 complementation groups¹⁰, so that these findings do not necessarily exclude a specific control of nervous system development by genes from this region. Even so, the results described here clearly demonstrate the efficacy of chromosome deletions as a tool with which to screen for neurogenic loci within the *Drosophila* genome.

We thank Sigrid Krien for technical assistance, Loring Craymer and A. Garcia-Bellido for mutant stocks, A. Garcia-Bellido, G. Jürgens, W. Michalke and P. A. Lawrence for discussions and for commenting on the manuscript, and the Deutsche Forschungsgemeinschaft (SFB 46) for financial support. F.J. holds an EMBO long-term fellowship.

3. Garcia-Bellido, A. & Moscoso del Prado, J. *Nature* **278**, 346–348 (1979).
4. Poulson, D. F. *Am. Nat.* **79**, 340–363 (1945).
5. Zalokar, M. & Erk, I. *Stain Technol.* **52**, 89–95 (1977).
6. Garcia-Bellido, A. & Santamaria, P. *Genetics* **88**, 469–486 (1978).
7. Garcia-Bellido, A. *Genetics* **91**, 491–520 (1979).
8. Poulson, D. F. in *Biology of Drosophila* (ed. Demerec, M.) 168–274 (Wiley, New York, 1950).
9. Bauer, V. *Zool. Jb.* **20**, 123–150 (1904).
10. Ripoll, P. & Garcia-Bellido, A. *Genetics* **91**, 443–453 (1979).
11. Lindsley, D. L. & Grell, E. H. *Genetic Variations of Drosophila melanogaster* (Carnegie Institute, Washington, 1968).
12. Bridges, C. B. *J. Hered.* **29**, 11–13 (1938).
13. Hayman, D. L. & Madder, R. H. *Drosoph. Inf. Serv.* **49**, 72 (1972).
14. Muller, H. J. *Genetica* **17**, 237–252 (1935).

Histone gene deficiencies and position–effect variegation in *Drosophila*

Gerald D. Moore, James D. Procunier, David P. Cross* & Thomas A. Grigliatti

Department of Zoology, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

Position–effect variegation, originally observed in insects, has since been demonstrated in plants and mammals. When cells bear a chromosomal rearrangement which juxtaposes a euchromatic gene near to heterochromatin, a fraction of the cells exhibit no expression of that gene. The resultant organism is a mosaic for activity of the rearranged gene. The degree of mosaicism can be modified; such factors as elevated developmental temperature, additional Y chromosome heterochromatin in the genome, and various modifier genes enhance the proportion of cells in which the gene is active¹. Although the phenomenon has been extensively documented, the underlying molecular mechanism of position–effect variegation remains unclear. The proportion of cells exhibiting gene inactivation increases with greater proximity of the rearranged gene locus to heterochromatin. It has been proposed that the variegating gene is inactivated by a ‘spreading effect’, or limited spatial diffusion of molecules from the adjacent heterochromatin². This explanation supposes that the juxtaposed gene locus is condensed into a transcriptionally inactive state, possibly by a phosphorylated histone protein subspecies characteristic of *Drosophila* heterochromatin³. Such a model leads to the prediction that an alteration in the amount of cellular histone protein would affect the expression of variegating genes. To test this prediction, we have investigated the effect of a reduction in the number of genes coding for histone protein on the degree of mosaicism observed in two variegating gene systems. Our results suggest that the amount of variegating gene expression is a function of histone gene multiplicity.

The histone genes of *Drosophila melanogaster* have been localised to a discrete region on the left arm of the second chromosome (polytene bands 39D3–E2)⁴. This region contains a nucleotide sequence coding for all five histone protein species, tandemly reiterated about 100 times⁵. Individuals whose genotypes contain single or triple histone clusters instead of the normal diploid complement are viable and phenotypically normal. Deficiencies in the left arm of the second chromosome have been generated, and characterised with respect to their breakpoints⁶. Five of these deficiency chromosome stocks were obtained; all have similar breakpoints to the left of the histone gene cluster. Two of the deficiencies, *Df(2L)1* and *Df(2L)12*, extend rightwards towards the histone gene cluster, but do not include it (Fig. 1). They serve as controls for any effect of deleting euchromatic segments to the left of the histone gene cluster. One deficiency, *Df(2L)84*, has its right breakpoint in the

Received 11 July; accepted 20 September 1979.

1. Wright, T. R. F. *Adv. Genet.* **15**, 261–395 (1970).
2. Poulson, D. F. *J. exp. Zool.* **83**, 271–325 (1940).

* Present address: Department of Neurosciences, McMaster University, Hamilton, Ontario Canada.

Table 1 Mean percentage of the wild-type amount of drosopterin in eyes of *In(1)w^{m4}/Y* F₁ flies from the cross: *+ / Y; CyO / Df(2L)♂♂ × w^{m4} / w^{m4}; + / + ♀♀*

F ₁ ♂ genotype	No. of histone gene clusters	% w ⁺ drosopterin	S _Y	P
<i>w^{m4} / Y; Df(2L)1 / +</i>	2	4	1.2	> 0.05
<i>w^{m4} / Y; CyO / +</i>	2	3	0.7	
<i>w^{m4} / Y; Df(2L)12 / +</i>	2	4	0.9	
<i>w^{m4} / Y; CyO / +</i>	2	3	0.5	> 0.05
<i>w^{m4} / Y; Df(2L)84 / +</i>	1-2	19	2.4	
<i>w^{m4} / Y; CyO / +</i>	2	6	0.7	
<i>w^{m4} / Y; Df(2L)65 / +</i>	1	9	2.1	< 0.05
<i>w^{m4} / Y; CyO / +</i>	2	3	0.4	
<i>w^{m4} / Y; Df(2L)161 / +</i>	1	24	3.2	
<i>w^{m4} / Y; CyO / +</i>	2	8	1.7	< 0.05
<i>w^{m4} / Y; + / +</i>	2	4	0.9	

F₁ progeny were reared at 17°C and allowed to age to 1-7 d post-eclosion. Approximately 50 flies of each type were decapitated by freezing and agitation. Single heads were crushed on cellulose chromatography plates (Eastman 13255) and pigments were separated with a 2:1 propanol: 1% NH₃ solution. The drosopterin spots were located, and relative levels of fluorescence measured, using a Zeiss microscope equipped with a UV light source and a photomultiplier. The fluorometer was standardised against the amount of drosopterin fluorescence from *w⁺* (Oregon-R strain) heads. Significance of difference in mean drosopterin amounts between *Df(2L)/+* and *CyO/+* siblings was tested using a *t*-test.

histone gene cluster. The proportion of the cluster which it deletes is uncertain. The remaining deficiencies, *Df(2L)65* and *Df(2L)161*, delete the entire histone gene cluster. The five deficiencies were tested on two chromosomal rearrangements which exhibit position-effect variegation. The first was *In(1)w^{m4}*, an inversion which relocates the *white⁺* gene from its normal position at the tip of the X chromosome to the centromeric heterochromatin. The *white⁺* gene is responsible for the formation of pigment granules in the pigment cells⁷. Individual cells of a *white* variegated, mosaic eye have either a normal complement of pigment granules, or are devoid of them⁸. A *w^{m4} / Y; + / +* eye contains about 4% of the wild-type amount of the red pigment drosopterin, when flies are raised at 17°C. The control deficiencies on the second chromosome (*Df(2L)1*, *Df(2L)12*) do not significantly alter this level (Table 1). *In(1)w^{m4}* flies heterozygous for the other three deficiencies which delete all, or a portion of the histone gene cluster, exhibit significantly elevated levels of eye drosopterin. Active expression of the *white⁺* gene occurs in a greater proportion of *In(1)w^{m4}* pigment cells with a single histone gene cluster than in those with the normal diploid complement.

The narrow eye phenotype associated with the mutation *Bar* is the result of a tandem duplication in the X chromosome region 16A (ref. 9). The rearrangement *B^{SV}Y* attaches this duplicated region to the heterochromatin of the Y chromosome¹⁰. As this transposed region is inactivated by position-effect variegation in a proportion of the presumptive ommatidial (eye facet) cells, *B^{SV}* flies exhibit a phenotype intermediate between the narrow eye of *Bar* and the normal, oval *Bar⁺* eye. In this case, suppression of the spreading effect should result in a narrower eye phenotype in *B^{SV}* flies, as the transposed duplication segment would be actively expressed in a greater proportion of presumptive ommatidial cells. This was observed in *B^{SV}* flies heterozygous for deficiencies of the histone gene cluster (Table 2). The effect of deficiency *Df(2L)84*, which deletes a portion of the histone gene cluster, is not as pronounced as that of deficiencies *Df(2L)65*, *161* which delete the entire cluster.

The results obtained using these two variegating gene systems confirm the prediction that a reduction in histone gene dosage will enhance active expression of variegating genes. Khesin and Liebovitch¹¹ obtained similar results using the variegating *white* allele associated with translocation *T(1;3)w^{eco}*. Their

methodology was seriously flawed, however, by constructing segmentally aneuploid histone gene deficiencies from translocation *T(Y;2)* chromosomes¹². In addition to deleting euchromatic portions of the chromosome, this technique produces poorly defined deficiencies and duplications of Y chromosome material. The Y chromosome contains regions which are potent suppressors of variegation¹³ and whose integrity in *T(Y;2)* chromosomes is questionable. We have tested a series of deficiencies, non-deficiencies and duplications of the histone gene cluster constructed from (Y;2) translocations. The translocated Y chromosomes vary widely and unpredictably in their ability to suppress or enhance variegation. Hence, we have confined our study to the use of deficiencies restricted to small euchromatic portions of chromosome 2. These deficiencies allow us to control for any effect of Y heterochromatin by utilising uniform Y chromosomes. Of the five deficiency chromosomes tested, four (*Df(2L)1*, *12*, *65* and *84*) were generated from a chromosome bearing *Tft1(2)74i* while *Df(2L)161* was imposed on a *cn bw* chromosome. Although it is possible that the two source chromosomes vary with respect to undefined variegation modifying loci, this seems unlikely given the similarity of modification observed with the *Df(2L)65* and *Df(2L)161* chromosomes.

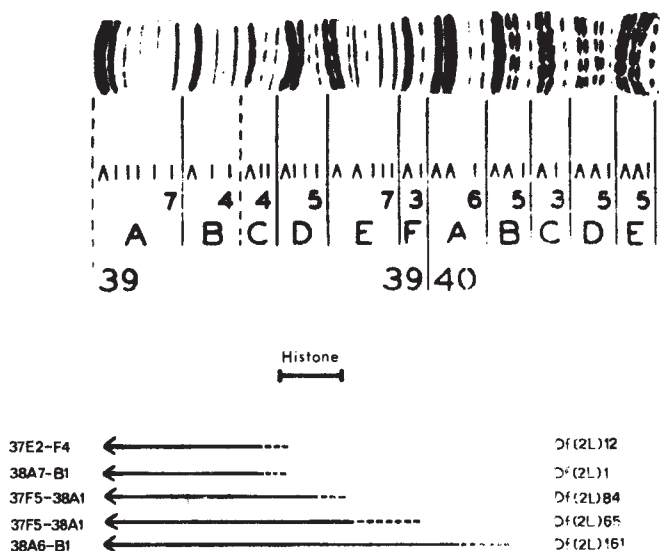


Fig. 1 A segment of the polytene chromosome map of *D. melanogaster* second chromosome showing the location of the histone gene cluster. The extent of each deficiency is indicated by a horizontal bar. Left breakpoints are listed; the right breakpoints fall within the broken portion of the bars. Cytological examination was originally performed by Wright *et al.*⁶ and has been reconfirmed in our laboratory.

Our results do not entirely eliminate the possibility that the observed suppression of position-effect variegation is due to the deletion of a gene tightly linked to the histone gene cluster, rather than deletion of the cluster itself. It should be noted, however, that heterozygotes for a control deficiency close to the left of the histone gene cluster (*Df(2L)1/+*, *Df(2L)12/+*) do not exhibit more active expression of either variegating gene than do the non-deficiency *CyO/+* sibling or *+ / +* controls. Flies heterozygous for the deficiency that does not extend to the right of the histone cluster (*Df(2L)84/+*), do exhibit more active expression of the variegating genes. Hence, if deletion of a gene outside the histone gene cluster is responsible for the

results obtained, it must be tightly linked to the left of the cluster.

Reduction in the number of histone genes has not been directly correlated to a decrease in the amount of cellular histone protein; indeed, it seems improbable that the reduction in histone protein amount is equivalent to the reduction in gene multiplicity. However, we interpret our results to suggest that compensation for the reduced histone coding capacity is incomplete, and that the level of cellular histone protein is partially reduced during the period when the transcriptional fate of the variegating genes is determined, since reduction in the coding capacity for histone protein markedly affected the level of variegating gene expression.

Table 2 Mean percentage of wild-type (B^+) eye surface in $+B^{SV}Y$ F_1 ♂ flies from the cross: $+Y; CyO/Df(2L)\delta\delta \times XX/B^{SV}Y; +/+ \varnothing\varnothing$

F_1 ♂ genotype	No. of histone gene clusters	% B^+ eye size	S_Y	P
$+B^{SV}Y; Df(2L)1/+$	2	53	1.9	<0.5
$+B^{SV}Y; Df; CyO/+$	2	47	1.9	
$+B^{SV}Y; Df(2L)12/+$	2	43	1.1	
$+B^{SV}Y; CyO/+$	2	48	1.2	>0.05
$+B^{SV}Y; Df(2L)84/+$	1-2	38	1.3	
$+B^{SV}Y; CyO/+$	2	44	1.6	<0.05
$+B^{SV}Y; Df(2L)65/+$	1	26	0.9	
$+B^{SV}Y; CyO/+$	2	46	1.1	$\ll 0.05$
$+B^{SV}Y; Df(2L)161/+$	1	18	0.7	
$+B^{SV}Y; CyO/+$	2	43	1.9	$\ll 0.05$

F_1 progeny were reared at 21 °C. Approximately 100 eyes of each type were sketched with the aid of a dissecting microscope equipped with an ocular micrometer. Outlines were cut out and weighed to estimate relative surface area. An ANOVA test revealed no significant difference between $CyO/+$ sibling values. $Df(2L)/+$ values were compared to the sibling $CyO/+$ values using a t -test.

Intensity of gene inactivation is proportional to the fraction of salivary gland chromosomes in which the variegating gene locus has been heterochromatinised¹⁴. Our results implicate histone proteins as agents of heterochromatinisation. The role of Y chromosome heterochromatin in suppressing position-effect variegation can be envisaged as that of a competitor for free histone protein, which would otherwise be available to condense variegating gene loci². Substances which affect the function of histone proteins in chromatin, such as sodium butyrate which inhibits histone deacetylation¹⁵, can also be expected to have an effect on variegating gene expression. Modification of position-effect variegation may be useful property for testing presumptive mutations of histone gene function and multiplicity.

We thank Dr J. Berger for the use of his quantitative microscopy facility, and Dr D. T. Suzuki for his helpful criticism. The work was supported by an NRC grant to Dr Suzuki and T. Grigliatti, and an NAHS grant to T.G.

Received 15 March; accepted 7 September 1979.

- Spofford, J. B. in *The Genetics and Biology of Drosophila* Vol. 1c (eds Ashburner, M. & Novitski, E.) 955-1018 (1976).
- Zuckerkind, E. *Biochimie* **56**, 937-954 (1974).
- Blumenfeld, M. et al. *Proc. natn. Acad. Sci. U.S.A.* **75**, 866-870 (1978).
- Pardue, M. L. et al. *Chromosoma* **63**, 135-151 (1977).
- Lifton, R. P. et al. *Cold Spring Harb. Symp. quant. Biol.* 1047-1051 (1977).
- Wright, T. R. F., Hodgetts, R. B. & Sherald, A. F. *Genetics* **84**, 267-285 (1976).
- Ziegler, I. *Adv. Genet.* **10**, 349-403 (1961).
- Shoup, J. R. *J. Cell Biol.* **29**, 223-249 (1966).
- Bridges, C. *Science* **83**, 210-211 (1936).
- Brosseau, G. E. *Jr Genetics* **45**, 979 (1960).
- Khesin, R. B. & Liebovitch, B. A. *Molec. gen. Genet.* **162**, 323-328 (1978).
- Lindsley, D. L., Sandler, L. et al. *Genetics* **71**, 157-184 (1972).
- Brosseau, G. E. *Jr Genetics* **50**, 237 (1964).
- Hartmann-Goldstein, I. J. & Wargent, J. M. *Chromosoma* **52**, 349-362 (1975).
- Candido, E. P. M., Reeves, R. & Davie, J. R. *Cell* **14**, 105-113 (1978).

Cis-active control of mouse β -galactosidase biosynthesis by a systemic regulatory locus

Franklin G. Berger & Kenneth Paigen

Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, New York 14263

Several higher organisms have been reported in which enzyme levels are determined genetically by sites located in close proximity to the corresponding structural genes. In several cases, these sites have been shown to act by controlling the rates of enzyme synthesis¹⁻⁴. *Cis* compared with *trans* action has been tested for those proximate regulatory sites controlling enzymes for which appropriate structural variants exist^{2,5-8}. The rate of synthesis of β -galactosidase in mouse tissues is under the control of a regulatory locus, *Bgl-s*, that is tightly linked to the enzyme structural gene^{4,9}; we have tested the *cis/trans* nature of *Bgl-s* action by analysis of the electrophoretic mobility of the enzyme from animals heterozygous for the appropriate regulatory and structural alleles. Our results indicate that *Bgl-s* acts *cis*, controlling the expression of the structural gene located on the same chromosome.

A genetic complex, termed [*Bgl*], mapping near the *Mod-1* locus on chromosome 9, contains within it elements governing the structure, development and rate of synthesis of β -galactosidase^{4,9-15}. The structural gene *Bgl-e* is defined by an electrophoretic polymorphism which affects the enzyme in all tissues⁹.

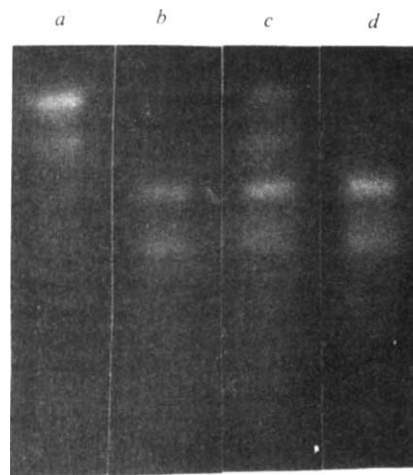


Fig. 1 Mobility patterns for kidney β -galactosidase as observed by enzyme activity stain. Kidneys from the appropriate animals were homogenised in 10 volumes (w/v) of 0.02 M imidazole-Cl, pH 7.4, containing 0.25 M sucrose. Homogenates were treated at pH 5.0 and 37 °C, and adjusted to a β -galactosidase concentration of 2 U ml⁻¹. Samples were subjected to isoelectric focusing on Ampholine PAG plates (pH 3.5-9.5) (LKB) for 2 h at 15 °C; β -galactosidase was stained by incubating the gels for 30 min in 0.1 M sodium citrate, pH 4.1, containing 0.05% Triton X-100 and 0.25 mM 4-methylumbelliferyl- β -D-galactoside (Sigma). Bands were observed and photographed under long wavelength ultraviolet light. a, Strain C57BL/6J, genotype *Bgl-e*^{b/b}_{s^{h/h}}; b, strain B10.C(47N)/Sn, genotype *Bgl-e*^{a/b}_{s^{h/h}}; c, F_1 (C57BL/6J $\times$ B10.C(47N)/Sn), genotype *Bgl-e*^{a/b}_{s^{h/h}}; d, F_1 (B10.C(47N)/Sn $\times$ B6.CBA-Trf^a*Bgl-s*^b), genotype *Bgl-e*^{a/b}_{s^{h/d}}. B10.C(47N)/Sn animals were obtained from Dr M. Cherry, Jackson Laboratory; B6.CBA-Trf^a*Bgl-s*^d animals were obtained from Dr Verne Chapman, Roswell Park Memorial Institute; C57BL/6J animals were purchased from the Jackson Laboratory.

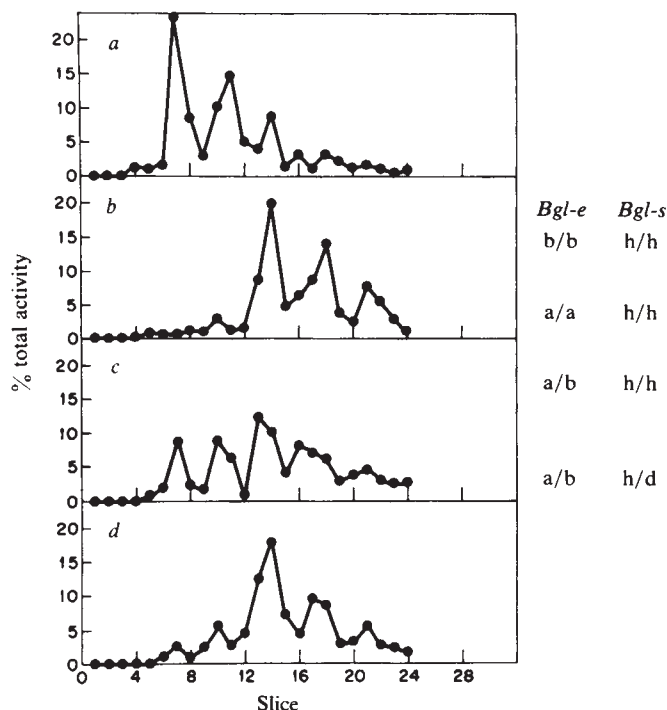


Fig. 2 Mobility patterns for kidney β -galactosidase as observed by direct assay. Kidney homogenates were prepared and isoelectric focusing was performed as described in Fig. 1 legend. After electrophoresis, gels were cut by hand into 2-mm slices, each slice was incubated for 2–4 h in 0.8 ml of 0.1 M sodium citrate containing 0.05% Triton X-100 and 0.25 mM 4-methylumbelliferyl- β -D-galactoside at 37 °C. After addition of 0.6 ml of 1 M sodium carbonate, fluorescence was measured in an Aminco fluorimeter⁹. The percentage of total activity recovered was plotted as a function of the number of slices from the cathode. (a), (b), (c) and (d) are the same as in Fig. 1 legend.

The allele coding for the more acidic form is designated $Bgl-e^a$ and that for the more basic form is designated $Bgl-e^b$. A systemic regulatory element, $Bgl-s$, controls β -galactosidase concentration in all tissues at all stages during development^{10–12}. It is tightly linked to $Bgl-e$ and controls the rate of β -galactosidase synthesis⁴. The high activity allele is designated $Bgl-s^h$ while the low activity allele is designated $Bgl-s^d$. Finally, the developmental programme for β -galactosidase synthesis in liver is genetically controlled by a temporal element within $[Bgl]$, termed $Bgl-t$, as well as by one or more elements separate from the complex^{4,13,15}. This last aspect of β -galactosidase regulation will not be considered further in this report.

Analysis of the β -galactosidase phenotype in more than 100 inbred mouse strains has made possible identification of three haplotypes of the $[Bgl]$ complex^{13,15}. The $[Bgl]^{B6}$ haplotype, exemplified by strain C57BL/6J, is $Bgl-e^b s^h$; the $[Bgl]^C$ haplotype, exemplified by strain BALB/cJ, is $Bgl-e^a s^h$; finally, the $[Bgl]^{D2}$ haplotype, exemplified by strain DBA/2J, is $Bgl-e^b s^d$. Table 1 summarises the biochemical and genetic relationships among the three haplotypes. Each haplotype is now available in the C57BL/6J or C57BL/10Sn genetic background, which seem to be equivalent with respect to effects on β -galactosidase expression¹⁵. The $[Bgl]^C$ haplotype of strain BALB/cJ has been transferred genetically into the C57BL/10Sn background to produce congenic strain B10.C(47N)/Sn; similarly the $[Bgl]^{D2}$ haplotype of strain CBA/J has been transferred genetically into the C57BL/6J background to produce congenic strain B6.CBA-Trf^a $Bgl-s^d$. The ability to construct animals heterozygous for the appropriate structural and regulatory elements of the $[Bgl]$ complex within a constant genetic background allows a test of the *cis* compared with *trans* nature of $Bgl-s$ action.

The mobility patterns after isoelectric focusing of kidney β -galactosidase from various animals were determined. Figure

1a shows the pattern for animals of strain C57BL/6J, which is homozygous for the $[Bgl]^{B6}$ haplotype. The multiple banding pattern represents charge heterogeneity resulting from unknown post-translation events affecting the product of a single structural gene^{14,16}. The same pattern is seen in C57BL/10Sn animals, which also carry the $[Bgl]^{B6}$ haplotype, and in strains carrying the $[Bgl]^{D2}$ haplotype, containing the $Bgl-e^b$ structural allele. Figure 1b shows the mobility pattern for the enzyme from congenic strain B10.C(47N)/Sn, which is homozygous for the $[Bgl]^C$ haplotype containing the structural gene coding for the more acidic enzyme. Figure 1c shows the enzyme pattern from F₁ progeny of a mating of strains C57BL/6J and B10.C(47N)/Sn; these animals are heterozygous for the $[Bgl]$ complex in that they carry the $[Bgl]^{B6}$ haplotype on one chromosome and the $[Bgl]^C$ haplotype on the other. Because these haplotypes differ only at $Bgl-e$ and not at $Bgl-s$ (Table 1), the pattern represents equal expression of both structural alleles. Figure 1d shows the pattern in F₁ progeny arising from a mating of strains B10.C(47N)/Sn and B6.CBA-Trf^a $Bgl-s^d$; these animals carry the $[Bgl]^C$ haplotype on one chromosome and the $[Bgl]^{D2}$ haplotype on the other and are therefore heterozygous for both the $Bgl-e$ structural and $Bgl-s$ regulatory elements of the complex (Table 1). They show predominant expression of the $Bgl-e^a$ structural type, which is *cis* to the high activity $Bgl-s^h$ regulatory allele within the complex. Because the relative expression of the two structural alleles in the heterozygote parallels the relative levels in the respective parental strains, the $Bgl-s$ genetic element governing these levels acts *cis*.

This conclusion is supported by slicing the gels and assaying the β -galactosidase activity in each slice. Figure 2 shows the results for the kidney enzyme from animals homozygous for the $[Bgl]^{B6}$ haplotype (Fig. 2a), animals homozygous for the $[Bgl]^C$ haplotype (Fig. 2b), F₁ heterozygotes carrying both the $[Bgl]^{B6}$ and $[Bgl]^C$ haplotypes (Fig. 2c), and F₁ heterozygotes carrying both the $[Bgl]^C$ and $[Bgl]^{D2}$ haplotypes (Fig. 2d). In the last case, the shift in mobility toward that of the $Bgl-e^a$ structural type, which is *cis* to the high activity $Bgl-s^h$ regulatory allele, supports the contention that the $Bgl-s$ locus is *cis*-active.

The assignment of the $Bgl-s$ element as *cis*-active is qualitative. Quantification of expression of each $Bgl-e$ allele in F₁ heterozygotes is complicated by the charge heterogeneity of each allelic product. This heterogeneity, along with the dimeric nature of β -galactosidase in the conditions of these experiments¹⁴, results in a complicated array of overlapping charge forms in $Bgl-e^{a/b}$ heterozygotes. This makes accurate determination of the level of expression of each $Bgl-e$ allele impossible. Attempts to remove the charge heterogeneity by chemical

Table 1 $[Bgl]$ haplotypes and their alleles at the $Bgl-e$ and $Bgl-s$ sites

$[Bgl]$ haplotype	Alleles*		Strains	Kidney β -galactosidase activity†	Relative rate of kidney β -galactosidase synthesis‡
	$Bgl-e$	$Bgl-s$			
B6	b/b	h/h	C57BL/6J	32.5 ± 5.8	1.33 × 10 ⁻⁴
			C57BL/10Sn	34.9 ± 5.6	—
C	a/a	h/h	BALB/cJ	28.8 ± 4.9	1.31 × 10 ⁻⁴
			B10.C(47N)/Sn	32.3 ± 4.2	1.50 × 10 ⁻⁴
D2	b/b	d/d	DBA/2J	13.4 ± 1.9	0.59 × 10 ⁻⁴
			CBA/J	12.6 ± 1.4	—
			B6.CBA-Trf ^a $Bgl-s^d$	12.6 ± 1.2	0.51 × 10 ⁻⁴

* The alleles of $Bgl-e$ (determined by measuring β -galactosidase electrophoretic mobility in polyacrylamide disc gels or on isoelectric focusing slab gels) and of $Bgl-s$ (determined by measuring enzyme activity in brain or kidney) carried by various strains have been listed previously⁹.

† Activities are expressed as μ mol of product formed per hour per gram wet weight of tissue $\pm$ s.d., using the fluorescent substrate 4-methylumbelliferyl- β -D-galactoside, as previously described⁹.

‡ Rates of synthesis were determined as previously described⁴ and are expressed as the ratio of c.p.m. incorporated into β -galactosidase to c.p.m. incorporated into total protein after brief labelling with ³H-leucine *in vivo*.

treatment (sulphydryl reagents, EDTA, periodate) or enzymatic treatment (neuraminidase, alkaline phosphatase, endoglycosidases) have been unsuccessful (ref. 14; F.G.B., unpublished work). Thus, quantitative measurement of expression of each structural allele must await more elaborate physicochemical techniques.

The lack of information concerning genetic fine structure of the *[Bgl]* region of chromosome 9 makes it difficult to speculate in detail on the mechanism of action of the *Bgl-s* element in regulating the rate of β -galactosidase synthesis. Our finding that *Bgl-s* acts *cis* is consistent with previous hypotheses that *Bgl-s* represents either a germ line change in the number of active β -galactosidase structural genes^{11,12}, or an alteration in a regulatory element adjacent to the structural gene¹¹. Although *Bgl-s* and the structural gene are inseparable by recombination⁹, the discordant distribution of the two determinants among inbred strains, as exemplified by the difference between the *[Bgl]^{B6}* and *[Bgl]^C* haplotypes, suggests that they are not identical sites. However, it has yet to be determined whether the *Bgl-s* site is located within the structural gene or is a closely linked element outside of it.

In summary, the *Bgl-s* locus, which determines the rate of β -galactosidase synthesis and is tightly linked to the structural gene within the *[Bgl]* complex on chromosome 9, seems to act *cis*, regulating the expression of the structural allele located on

the same chromosome; it is therefore unlikely that it codes for a diffusible regulatory molecule. Other *cis*-active elements, mapping in close proximity to their respective structural genes, have been identified in higher organisms^{2,5-8}. One of these, the *Gus-r* locus controlling androgen induction of β -glucuronidase in mouse kidney, has been shown to act at the level of enzyme synthesis². The *Bgl-s* locus is interesting in that it is the first systemic regulator of enzyme synthesis for which *cis/trans* action has been testable.

This study was supported in part by USPHS grant GM-19521. We thank Drs M. Cherry and V. Chapman for mice.

Received 20 June; accepted 24 September 1979.

1. Doyle, D. & Schimke, R. T. *J. biol. Chem.* **244**, 5449-5459 (1969).
2. Swank, R. T., Paigen, K. & Ganschow, R. E. *J. molec. Biol.* **81**, 225-243 (1973).
3. Ganschow, R. E. in *Isozymes* (ed. Markert, C. L.) 633-647 (Academic, New York, 1975).
4. Berger, F. G., Paigen, K. & Meisler, M. H. *J. biol. Chem.* **253**, 5280-5282 (1978).
5. Schwartz, D. *Genetics* **47**, 1609-1615 (1962).
6. Dickson, W. J. *Dev. Biol.* **42**, 131-140 (1975).
7. Peterson, A. C. & Wong, G. C. *Nature* **276**, 267-269 (1978).
8. McCarron, M. *et al. Genetics* **91**, 275-293 (1979).
9. Breen, G. A. M., Lusi, A. J. & Paigen, K. *Genetics* **85**, 73-84 (1977).
10. Lundin, L.-G. & Seyedzadani, R. *Biochem. Genet.* **10**, 351-361 (1973).
11. Felton, J., Meisler, M. & Paigen, K. *K. biol. Chem.* **249**, 3267-3272 (1974).
12. Meisler, M. H. *Biochem. Genet.* **14**, 921-932 (1976).
13. Paigen, K., Meisler, M., Felton, J. & Chapman, V. M. *Cell* **9**, 533-539 (1976).
14. Lusi, A. J., Breen, G. A. M. & Paigen, K. *J. biol. Chem.* **252**, 4613-4618 (1977).
15. Berger, F. G., Breen, G. A. M. & Paigen, K. *Genetics* (in the press).
16. Berger, F. G. & Lusi, A. J. *Biochem. Genet.* **16**, 1007-1014 (1978).

Polymorphisms of human γ -globin genes in Mediterranean populations

Peter F. R. Little*, Robert Williamson*, Gillian Annison*, Richard A. Flavell†, Ernie De Boer‡, L. F. Bernini§, Sergio Ottolenghi§, Giuseppe Saglio|| & Umberto Mazza||

* Department of Biochemistry, St Mary's Hospital Medical School, University of London, London W2, UK

† Laboratory of Biochemistry, University of Amsterdam, PO Box 60,000, 1005 GA Amsterdam, The Netherlands

‡ Instituut voor Anthropogenetica der Rijksuniversiteit, Sylvanus Laboratoria, Wassenaarse-weg 72, Leiden, The Netherlands

§ Istituto di Patologia Generale, Università degli Studi, 20133 Milan, Italy

|| Istituto di Medicina Interna, Università di Torino, Turin, Italy

During normal human fetal development, two γ -globin genes are expressed to give γ -globin chains, one of which contains an alanine residue and the other a glycine residue at amino acid position 136 ($^A\gamma$ and $^G\gamma$, respectively)¹. Recently, a human γ -globin chain mutant has been described in HbF_{Sardinia} in which threonine replaces isoleucine at position 75 (ref. 2). This haemoglobin is frequently found in varying levels in Italian patients with β -thalassaemia (29 out of 32 patients), and in normal fetal cord blood in some populations (9 out of 19)³. In England the proportion of infants expressing this variant (usually at low levels but occasionally up to 40% of the total fetal globin) is approximately one in five. The number of γ -globin gene loci has long been controversial. The two chains $^A\gamma$ and $^G\gamma$ are non-allelic and therefore the minimum possible number of genes is two. The change in $^A\gamma/^G\gamma$ chain ratio from 0.3 to 1.5 as fetal development proceeds led Huisman and Schroeder to propose four γ -globin genes per haploid genome^{4,5}. Direct molecular hybridisation of γ -globin DNA sequences gave data supporting the two-gene hypothesis⁶⁻⁸. Because of its widespread occurrence, it has been suggested that the γ -chain in HbF_{Sardinia} is coded by a third structural gene, $^T\gamma$, non-allelic with $^A\gamma$ and $^G\gamma$ (ref. 3). We show here by direct restriction analysis of the γ -globin genes that people expressing HbF_{Sardinia} have two γ -globin genes per haploid set, and therefore that the γ -chain in HbF_{Sardinia} is coded for by an allele of either the $^A\gamma$ - or $^G\gamma$ -globin gene.

Using the techniques of restriction enzyme analysis, we have recently studied the number and arrangement of γ -globin genes in humans. The map obtained is shown in Fig. 1. There are two γ -globin genes per haploid genome, separated by 3.5 kilobases. Each gene is split into at least two segments by an intervening sequence (intron)¹⁰ at some position along the DNA coding for amino acids 100-121, the same position as for the human β - and δ -globin genes³. Other investigators^{11,12} have also shown by molecular cloning that the $^A\gamma$ -globin gene has two introns at precisely the same positions as those in the human β -globin

Table 1 Samples used in the study of γ -globin gene number in Mediterranean populations

Description	Origin	DNA sample source	Level of HbF _{Sardinia}
1. Normal	Dutch	Placenta	Unknown
2. Normal	British	Placenta	Unknown
3. Normal	Dutch	Cord blood	0%
4. Normal	Dutch	Cord blood	20%
5. Lepore	Southern Italy	Spleen	0%
6. β^0 thalassaemia	Southern Italy	Spleen	0%
7. β^+ thalassaemia	Northern Italy	Spleen	8%
8. β^0 thalassaemia	Sardinia	Spleen	20%
9. β^0 thalassaemia	Italy	Spleen	20%
10. β^+ thalassaemia	Southern Italy	Spleen	25%
11. β^0 thalassaemia	Northern Italy	Spleen	30%

The proportion of HbF_{Sardinia} in cord blood samples of normal humans and peripheral blood samples of thalassaemia patients was estimated by separation of γ chains by CM-cellulose chromatography and subsequent tryptic digestion as detailed in ref. 17.

gene. In our previous study⁹, we had no information on the level of expression of HbF_{Sardinia} in the individuals studied. Therefore, we still did not know whether the gene specifying the so-called $^T\gamma$ chain was a polymorphism of γ -globin gene number within the human population or whether the $^T\gamma$ -globin gene was allelic

with either the $^A\gamma$ - or $^G\gamma$ -globin gene, the two genes defined on the map we obtained⁹. The techniques of restriction enzyme site mapping¹³ allow a simple test for this possibility.

We have made DNA from the spleens of seven β -thalassaemics and two non-thalassaemics with known levels of HbF_{Sardinia} in their peripheral blood. The various samples used are detailed in Table 1. We have analysed the γ -globin gene region of these DNA samples. Briefly, this analysis involves digestion of the DNA with restriction endonucleases, denaturation and separation of DNA fragments according to size by electrophoresis on 1.0% or 1.2% agarose gels, transfer of DNA from gel to nitrocellulose filter sheets by 'blotting' according to the method of Southern¹³, and detection of the fragments containing γ -globin gene sequences by hybridisation with ³²P-labelled pHyGI and subsequent autoradiography. pHyGI is a pCRI recombinant plasmid which contains a fragment of 500 base pairs of double-stranded $^G\gamma$ -globin cDNA prepared from mRNA γ (ref. 14).

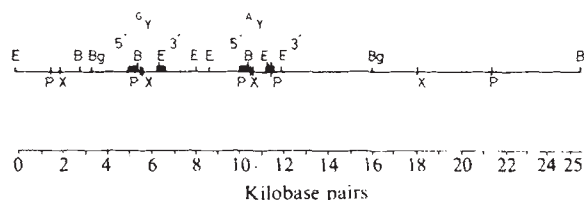


Fig. 1 The physical map of the human fetal globin genes. The position of each gene is marked by a filled box. Note that only those restriction enzyme sites that generate fragments containing part or all of the γ -globin genes are shown on this map. Although the genes are shown as split by a single intron, it is not possible to exclude the existence of further, small, introns in the $^G\gamma$ -globin gene. The small intron in the $^A\gamma$ -globin gene is not shown. The map is derived from ref. 9. E, *EcoRI*; B, *BamHI*; Bg, *BglII*; P, *PstI*; X, *XbaI*.

We have examined the γ -globin fragment patterns generated by *EcoRI* for all the samples in Table 1, and have also used the enzymes *BglII*, *BamHI* (samples 3, 4, Table 1) and *KpnI* (samples 5–11, Table 1). In all cases the size and number of fragments are identical to those given in Table 2, generated from normal individuals (samples 1, 2) shown previously to contain only two γ -globin genes per haploid genome⁹. These data strongly suggest that there are only two γ -globin genes per haploid genome present in individuals expressing HbF_{Sardinia}. An alternative explanation would be that a third γ -globin gene exists coding for the ' $^T\gamma$ ' chain, but that in each digest it is present on a DNA fragment which co-migrates with one of the $^A\gamma$ - or $^G\gamma$ -globin gene fragments. We consider this unlikely; the fragment pattern is the same for DNA digested with four enzymes, whether they give separate $^A\gamma$ and $^G\gamma$ pieces (*EcoRI* and *BamHI*), a fragment bridging the $^G\gamma$ - and $^A\gamma$ -globin genes (*BamHI*), a fragment containing both genes (*BglII*) or a fragment containing the entire $^G\gamma^A\gamma\delta\beta$ complex (*KpnI*, see ref. 15). The only reasonable objection to this would be a reduplication of the $^G\gamma^A\gamma\delta\beta$ locus; this is inconsistent with the available evidence on the inheritance of β -globin variants.

It could be argued that a putative $^T\gamma$ -globin gene would not cross-hybridise well with the $^G\gamma$ cDNA plasmid probe used here due to extensive third position codon changes in the coding sequences. It would then be possible to miss new $^T\gamma$ -globin gene-specific fragments because they would not be stable in the washing conditions used. To eliminate this possibility, *EcoRI* digests of three DNA samples from β -thalassaemic patients with 0%, 8% and 20% HbF_{Sardinia}, and DNA from a non-thalassaemic control known to contain only two γ -globin genes were hybridised with pHyGI and the filters subjected to increasingly stringent salt washes ranging from $3 \times \text{SSC}$ ($\text{SSC} = 0.15 \text{ M NaCl}$, $0.015 \text{ M Na citrate}$) to $0.01 \times \text{SSC}$ at 65°C (Fig. 2). Washing of

filters at low salt concentrations (in this case, $0.1 \times \text{SSC}$) causes mismatched hybrids to melt, and as a result only hybrids between γ -globin gene sequences (both $^A\gamma$ and $^G\gamma$) and the γ cDNA plasmid probe are seen. Filters washed only with high salt (greater than $0.1 \times \text{SSC}$) show heterologous hybridisation (for example, with the β -globin gene sequences) in addition to the $^A\gamma$ - and $^G\gamma$ -globin gene hybridisation. In all conditions, the γ -globin gene-specific fragments seen are identical. This demonstrates that no third globin gene distantly related to that coding for $^G\gamma$ -globin exists in persons expressing HbF_{Sardinia} which would be absent in those people not expressing this protein.

The gene coding for the γ -globin chain of HbF_{Sardinia} must, therefore, be allelic with either the $^A\gamma$ - or the $^G\gamma$ -globin gene. There is evidence^{16,17} that the threonine residue at position 75 is present only in γ -globin chains that contain alanine at position 136 and we therefore conclude that the γ -globin chain of HbF_{Sardinia} is an allele of the $^A\gamma$ gene.

The high level of occurrence of HbF_{Sardinia} remains to be explained. In one study³, the incidence of HbF_{Sardinia} in β -thalassaemics in Italy was 90%, compared with only 40% in normal Italians. It is possible that the HbF_{Sardinia} mutation is in linkage disequilibrium with either the β -thalassaemic gene or some other gene. It is not known whether the Ile 75 $\rightarrow$ Thr 75 substitution in HbF_{Sardinia} alters the physiological properties of the haemoglobin molecule. It is therefore impossible to distinguish between mechanisms of selection, drift or linkage disequilibrium in accounting for the high frequency of this haemoglobin molecule. Indeed, the presence of the fixed mutations in $^A\gamma$ and $^G\gamma$ in the otherwise identical γ -globin chains seems to be an earlier example of a similar phenomenon.

As duplicated γ -globin genes have been demonstrated in the pig-tailed macaque (*Macaca nemestrina*)¹⁸, orang-utan, gorilla and chimpanzee^{19,20}, we conclude that the duplication occurred at least 30 Myr ago²¹. It is remarkable that the two γ -globin genes have remained so similar during this time; some mechanism may exist to maintain sequence homology between these two genes.

Unequal cross-over events have been invoked to explain the conservation of multi-copy genes such as those for ribosomal RNAs^{22–24}. This involves a contraction/expansion of gene numbers by unequal cross-over events and re-amplification by a further cross-over between two gene families of different sizes. In contrast to the ribosomal RNA genes, the only simple way in which a single γ -globin gene may be amplified is by a cross-over with a chromosome containing three γ -globin genes. This makes unequal cross-over a less attractive mechanism for sequence conservation. In addition, data on 810 humans showed that

Table 2 Sizes of DNA fragments containing γ -globin genes generated by four restriction endonucleases

Enzyme	Fragment sizes (kilobase pairs)
<i>BamHI</i>	15, 5.0, 2.6
<i>EcoRI</i>	6.5, 2.5, 1.65, 0.65
<i>BglII</i>	13
<i>KpnI</i>	46

The human DNA used has been shown by restriction endonuclease mapping to contain only two γ -globin genes per haploid genome¹⁷. The relative positions of each fragment are as given in Fig. 1 or ref. 15.

none of them synthesised only $^A\gamma$ - or only $^G\gamma$ -globin chains²⁵. These experiments were not designed to detect 'heterozygotes' in which one chromosome had duplicated γ genes and the other had only a single γ -globin gene. From these data we calculate that the frequency of chromosomes carrying only a single γ -globin gene is less than 0.06 ($P = 0.05$).

It would be interesting to examine a large number of HbF_{Sardinia} γ -globin chains at position 136. If there is a mechanism for spreading mutational changes between the $^A\gamma$

and γ genes it would be predicted that $\gamma^{75\text{Thr}}$ should be found associated with $\gamma^{136\text{Gly}}$. The frequency of this occurrence would be an indicator of the rate of exchange of genetic information between γ and ϵ . A precise study of the HbF_{Sardinia} mutation would provide an interesting insight into mechanisms of gene conservation.

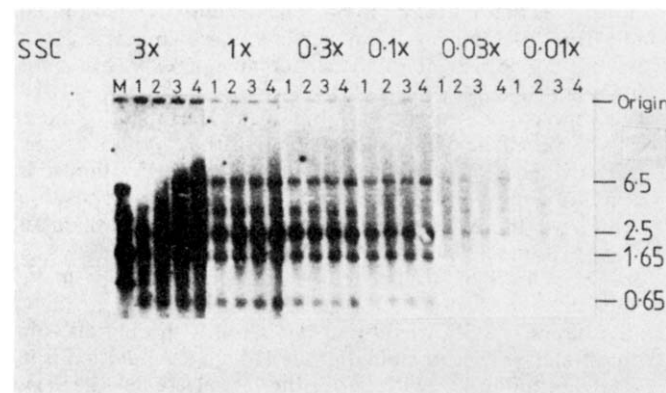


Fig. 2 The pattern of γ -gene fragments generated by *EcoRI*. DNA digestion, gel electrophoresis and hybridisation were carried out as in ref. 17. After hybridisation, duplicate filters were washed in various concentrations of SSC buffer (1 × SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.5, 0.1% (w/v) SDS). The weakly hybridising bands at approximately 4–5 kilobases are due to cross-hybridisation between the γ -globin probe and β - and ϵ -globin gene sequences. Samples used expressed different levels of HbF_{Sardinia}: 1, 20%; 2, 8%; 3, 0%; 4, control DNA; and correspond, respectively, to samples 5, 7, 8 and 2 in Table 1. Sizes are in kilobases.

We thank Dr Alec Jeffreys for helpful discussions, and Caroline Rogers for help with the manuscript. This work was supported by grants to R.W. from the MRC and NIH (1R01AM20125-01A1), and to R.A.F. from the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Science (ZWO). A grant from NATO made the collaboration possible.

Received 7 June; accepted 28 September 1979.

- Schroeder, W. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **60**, 537–544 (1968).
- Grifoni, V., Kamuzoro, H., Lehmann, H. & Charlesworth, D. *Acta haemat.* **53**, 347–355 (1975).
- Rico, G. *et al. Hum. Genet.* **32**, 305–313 (1976).
- Huisman, T. H. J., Schroeder, W. A., Bannister, W. H. & Grech, J. L. *Biochem. Genet.* **7**, 131–139 (1970).
- Schroeder, W. A. *et al. Nature new Biol.* **244**, 89–90 (1973).
- Old, J. *et al. Cell* **8**, 13–18 (1976).
- Ramirez, F., O'Donnel, J. V., Nathan, C. & Bank, A. *J. clin. Invest.* **58**, 1475–1481 (1976).
- Mitchell, G. J. & Williamson, R. *Nucleic Acids Res.* **4**, 3557 (1977).
- Little, P. F. R., Flavell, R. A., Kooter, J. M., Anisson, G. & Williamson, R. *Nature* **278**, 227–231 (1979).
- Gilbert, W. *Nature* **271**, 501 (1978).
- Smithies, O. *et al. Science* **202**, 1284–1289 (1978).
- Blattner, F. R. *et al. Science* **202**, 1279–1284 (1978).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Little, P. *et al. Nature* **273**, 640–643 (1978).
- Bernards, R., Little, P. F. R., Anisson, G. A., Williamson, R. & Flavell, R. A. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Schroeder, W. A. *et al. J. clin. Invest.* **63**, 268–275 (1979).
- Saglio, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 3420–3424 (1979).
- Nute, P. & Stamatojanopoulos, G. *Nature new Biol.* **229**, 145 (1971); *Blood* **38**, 108–115 (1971).
- De Jong, W. W. *Biochim. biophys. Acta* **251**, 217–226 (1971).
- Huisman, T. H. S. *et al. Biochem. Genet.* **10**, 309–318 (1972).
- Simons, E. L. *Ann. N.Y. Acad. Sci.* **167**, 319–331 (1969).
- Smith, G. P. *Cold Spring Harb. Symp. quant. Biol.* **38**, 507–513 (1973).
- Smith, G. P. *Science* **191**, 528–535 (1976).
- Brown, D. D. & Sugimoto, K. *Cold Spring Harb. Symp. quant. Biol.* **38**, 501–505 (1973).
- Huisman, T. H. J., Schroeder, W. A., Felice, A., Powars, D. & Ringleham, B. *Nature* **265**, 63–65 (1977).

Relationships between sister chromatid exchange and mutagenicity, toxicity and DNA damage

Matthews O. Bradley*, Ih Chang Hsu & Curtis C. Harris

Human Tissue Studies Section, Laboratory of Experimental Pathology, National Cancer Institute, Bethesda, Maryland 20205

Reciprocal exchanges of DNA in sister chromatids (SCEs) are induced by various carcinogens and mutagens^{1–5}, although the quantitative relationship between the number of mutations and SCEs induced varies among chemicals⁵. Nevertheless, the analysis of SCE production by various agents is often proposed as a sensitive and quantitative assay for genetic damage of the sort leading to mutation and cancer. In V-79 Chinese hamster cells we have been measuring DNA damage by alkaline elution, mutation induction as detected by 6-thioguanine resistance, and cytotoxicity as detected by colony formation for different physical and chemical agents. Some of the agents produced varying forms of DNA damage but undetectable increases in either mutation or toxicity. We report here that some undetectably mutagenic and/or toxic agents produce increases in SCE frequency and that DNA single-strand breaks, DNA–DNA interstrand cross-links, and DNA–protein cross-links are not necessary for SCE.

The toxic, mutagenic and DNA-damaging properties of the agents used here are summarised in columns (1)–(7) of Table 1. All the data in Table 1 were obtained from the same line of V-79-4 previously described^{6,7}. Toxicity was measured by the loss of cell plating efficiency^{6,7}, mutation was measured as resistance to 6-thioguanine^{6,7} over a complete dose range that inactivated up to 90% of the cells, and DNA single-strand breaks, DNA–protein cross-links and DNA–DNA cross-links were measured by alkaline elution^{8–10}. Both *cis*- and *trans*-Pt(II) diammine dichloride (PDD) make DNA–DNA and DNA–protein cross-links, but the *cis* isomer is highly mutagenic in a dose-dependent manner whereas the *trans* isomer is not detectably mutagenic over a dose range of 25–450 μ M (ref. 8). Both the fluorescent lamp and the sunlamp cause mutation and toxicity as well as DNA single-strand breaks and DNA cross-links^{6,10,11}. If a filter of *p*-aminobenzoic acid is placed between the lamps and the cells, only light of wavelength >345 nm passes through to the cells. At the doses used, this light produced DNA single-strand breaks and DNA–protein cross-links but no detectable mutation or toxicity¹⁰. Hydrogen peroxide produces both toxicity and DNA single-strand breaks but is not detectably mutagenic even at doses up to 530 μ M (ref. 12).

In these experiments the doses of the toxic agents were chosen to give approximately 10–45% survival (exact value given in Table 1). The SCE frequency was measured in V-79 cells by the technique described by Perry and Wolff¹³. Two flasks of the V-79 cells (5×10^5 cells per 25-cm² flasks in log growth) in each group were treated with the doses shown in Table 1. Immediately after treatment the medium was changed to one containing 10 μ M bromodeoxyuridine. The cells were kept at 37 °C in the dark for two cycles of DNA replication before treatment with colcemid (0.1 μ g ml^{−1}). Preliminary growth curves were first measured to determine the time after treatment for two population doublings to occur. The mitotic cells were collected by trypsinisation 2 h after the start of colcemid treatment. Drops of cells in fixative were placed on clean microscope slides and allowed to dry in air. The air-dried preparations were stained by the fluorochrome plus Giemsa procedure¹³ and scored for SCE by one person who had no prior knowledge of the treatments. At least 60 metaphases were scored for each treatment group.

* To whom correspondence should be addressed. Present address: Merck Institute for Therapeutic Research, Merck Sharp & Dohme Research Laboratories W44-1, West Point, Pennsylvania 19486.

The results of the SCE measurements are shown in columns (8) and (9) of Table 1. The statistical significance of the results was determined by the Wilcoxon rank sum test¹⁴, a non-parametric method of analysis. *trans*-PDD, which is not detectably mutagenic, produces almost as many SCEs as does the highly mutagenic *cis*-PDD. As neither of these drugs produce DNA single-strand breaks, SCEs must be due to some other lesion, such as DNA-DNA and/or DNA-protein cross-links (Table 1). Although approximately equal numbers of SCEs are made by the two drugs at these concentrations, *trans*-PDD produces approximately 20-fold higher levels of DNA-protein cross-linking than does *cis*-PDD but equal numbers of DNA-DNA cross-links⁸. Therefore, in this instance, a closer quantitative relationship exists between DNA-DNA cross-links and SCEs than between DNA-protein cross-links and SCEs.

The radiation emitted from the sunlamp (General Electric 275-W sunlamp) produces many SCEs in V-79 cells (Table 1). The radiation was filtered with 3 cm of a 5-g l⁻¹ solution of potassium hydrogen phthalate to allow only wavelengths >305 nm to pass through. The sunlamp has a broad emission spectrum in the near UV and visible regions, with peaks at the mercury emission lines¹⁵. In these conditions, the sunlamp causes mutagenicity, toxicity, DNA-protein cross-links and DNA single-strand breaks^{10,11}. However, when a second filter of *p*-aminobenzoic acid is used to block wavelengths below 345 nm, the sunlamp radiation is no longer either mutagenic or toxic, but still produces numbers of SCEs. Thus, SCEs can be formed in conditions that produce neither detectable toxicity nor mutagenicity.

The fluorescent lamp radiation induces small but significant ($P < 0.01$) increases in SCE frequency (Table 1). The *p*-aminobenzoic acid filter reduces the mutagenicity and toxicity of the light to undetectable levels whereas the number of SCEs is slightly decreased although still significantly higher than the controls ($P < 0.05$). Again, toxicity and mutagenicity are not correlated with SCE frequency.

Hydrogen peroxide produces toxicity and large numbers of DNA single-strand breaks but no detectable mutagenicity².

Nevertheless, it causes significant increases in the SCE frequency, as seen in Table 1.

All the agents except *cis*- and *trans*-PDD make single-strand breaks and/or alkali-labile lesions in DNA. These platinum compounds produce the greatest increase in the frequency of SCEs, so single-strand DNA breaks are not required for SCE production. Hydrogen peroxide and both the filtered and unfiltered fluorescent lamp and sunlamp make SCEs but no detectable DNA-DNA cross-links, suggesting that these lesions are not uniquely involved in SCE formation. The DNA-protein cross-link is produced by all these agents except hydrogen peroxide. Therefore, none of the three lesions is made by all the agents, even though SCEs are made by all of them. These observations imply that none of the lesions examined is necessary for SCE production, although each of them, together with other lesions, could be sufficient for SCE formation. Of course, another lesion(s), not measured in these studies, could be uniquely involved in SCE formation.

The data presented here imply that correlations between SCE frequency and mutagenicity or toxicity are not necessarily close. Although it is difficult to be certain that an agent will not be weakly mutagenic in any conditions, nevertheless, given the limitations of the V-79 assay, four of the treatments in Table 1 are not detectably mutagenic but increase SCE frequencies. Resistance to 6-thioguanine as an assay for mutant colonies should score for many kinds of genetic or epi-genetic alterations in the enzyme hypoxanthine-guanine phosphoribosyl transferase. Although other genetic markers might give different quantitative results, we suggest that the qualitative conclusions would be similar.

The findings that *trans*-PDD and the filtered sunlamp cause large increases in SCE but no detectable mutagenicity suggest that the lesions causing SCEs in these cases differ from those causing 6-thioguanine-resistant mutations. Toxicity and SCEs are not necessarily correlated, as previously suggested by Wolff *et al.*⁴, because the filtered sunlamp and fluorescent lamp both increase SCE frequency but are not detectably toxic.

Recent work by Kinsella and Radman¹⁶ supports our contention that increased SCE frequency is not necessarily equivalent

Table 1 Relationships between sister chromatid exchanges, mutagenicity, toxicity and DNA damage

Agent	Quantity of agent	% survival (±s.e.m.)	Mutant frequency relative to control*	DNA Single-strand breaks and/or alkali-labile lesions†	DNA-Protein cross-links‡	DNA-DNA cross-links‡	SCEs per chromosome (±s.e.m.)	Significance compared with controls by Wilcoxon rank sum test	Refs for data in columns (3)-(7)
Control	—	100 ± 4.8	1	—	—	—	0.351 ± 0.23	—	—
<i>trans</i> -Pt (II) diammine dichloride	250 µM	16 ± 1.0	1	±‡	++	+	2.911 ± 0.131	$P < 0.01$	8
<i>cis</i> -Pt (II) diammine dichloride	10 µM	11 ± 0.5	40	—	+	+	3.960 ± 0.200	$P < 0.01$	8
GE 275-W sunlamp	2.4 × 10 ⁵ Jm ⁻²	45 ± 3.6	23	++	+	—	1.772 ± 0.135	$P < 0.01$	10,11
Sunlamp plus <i>p</i> -aminobenzoic acid filter	1.8 × 10 ⁵ Jm ⁻²	100 ± 5.4	1	+	+	—	1.556 ± 0.139	$P < 0.01$	10,11
Sylvania F15T8 cool white fluorescent lamp	5.9 × 10 ⁴ Jm ⁻²	35 ± 3.2	53	++	+	—	0.580 ± 0.034	$P < 0.01$	6,10
Fluorescent lamp plus <i>p</i> -aminobenzoic acid filter	3.2 × 10 ⁴ Jm ⁻²	100 ± 3.2	1	+	+	—	0.452 ± 0.019	$P < 0.05$	6,10
Hydrogen peroxide	353 µM	20 ± 1.6	1	++	—	—	0.616 ± 0.048	$P < 0.01$	12

Per cent survival was determined as the decrease in plating efficiency relative to the controls. The control plating efficiency varied between 75 and 95%.

* The numbers are the mutant frequency of the experimental group divided by the mutant frequency of the controls. Mutant frequency is defined as the number of colonies resistant to 6-thioguanine per survivor in appropriate expression and selection conditions as previously described^{6,7}. † Indicates that the treatments were not detectably mutagenic as defined by the lack of a dose-response relationship. The mutant frequencies of the various controls were within the limits of $1.3 \pm 0.9 \times 10^{-6}$.

‡ The symbols are: —, no lesions detectable; ±, barely detectable lesions; +, small numbers of lesions; ++, large numbers of lesions.

‡ The quantitative significance of the small number of apparent breaks has not been determined.

to increased mutant frequency. These workers have shown that the non-mutagenic tumour promoter, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) induces SCEs, whereas the non-promoting derivative 4-*O*-methyl TPA does not. Further support comes from the finding that saccharin also induces SCEs in both hamster and human cells¹⁷, although it is considered to be non-mutagenic^{18,19}. From these and our own results, we conclude that, although the analysis of SCEs can often be very useful, definitive interpretations of SCE data await a molecular mechanism for their formation.

Received 11 April; accepted 31 August 1979.

1. Latt, S. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3162-3166 (1974).
2. Kato, H. *Expl Cell Res.* **85**, 239-247 (1974).
3. Perry, P. & Evans, H. J. *Nature* **258**, 121-125 (1975).
4. Wolff, S., Rodin, B. & Cleaver, J. E. *Nature* **265**, 347-349 (1977).
5. Carrano, A. V., Thompson, L. H., Lindl, P. A. & Minkler, J. L. *Nature* **271**, 551-553 (1978).
6. Bradley, M. O. & Sharkey, N. A. *Nature* **266**, 724-726 (1977).
7. Bradley, M. O. & Sharkey, N. A. *Nature* **274**, 607-608 (1978).
8. Zwelling, L. A., Bradley, M. O., Sharkey, N. A., Anderson, T. & Kohn, K. W. *Mutat. Res.* **67**, 271-280 (1979).
9. Kohn, K. W., Erickson, L. C., Ewig, R. A. & Friedman, C. A. *Biochemistry* **15**, 4629-4637 (1976).
10. Bradley, M. O., Erickson, L. C. & Kohn, K. W. *Biochim. biophys. Acta* **520**, 11-20 (1978).
11. Erickson, L. C., Bradley, M. O. & Kohn, K. W. *Biochim. biophys. Acta* (submitted).
12. Bradley, M. O., Erickson, L. C. & Kohn, K. W. *Mutat. Res.* (submitted).
13. Perry, P. E. & Wolff, S. *Nature* **251**, 156-158 (1974).
14. Colton, T. *Statistics in Medicine*, 221-223 (Little, Brown and Company, Boston, 1974).
15. Jacobson, E. O. *et al. Mutat. Res.* **51**, 61-67 (1978).
16. Kinsella, A. & Radman, M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6149-6153 (1978).
17. Wolff, S. & Rodin, B. *Science* **200**, 543-545 (1978).
18. Stolz, D. R., Stavric, B., Klassen, R., Bendall, R. O. & Craig, J. J. *envir. Path. Tox.* **1**, 139-146 (1977).
19. Leonard, A. & Leonard, E. D. *J. envir. Path. Tox.* **2**, 1047-1053 (1979).

Intranuclear injection of anti-actin antibodies into *Xenopus* oocytes blocks chromosome condensation

D. Rungger

Department of Animal Biology, University of Geneva, 154, Route de Malagnou, 1224 Chêne-Bougeries, Switzerland

E. Rungger-Brändle, C. Chaponnier & G. Gabbiani

Department of Pathology, University of Geneva, 40, Boulevard de la Cluse, 1211 Geneva 4, Switzerland

The role of contractile proteins in the structural organisation of the interphase nucleus and of metaphase chromosomes is largely unknown. Actin has been found in interphase nuclei of different species, especially in association with condensed chromatin¹⁻⁶. In the germinal vesicle (nucleus) of *Xenopus* oocytes, actin has been localised in the nuclear gel supporting the chromosomes and the extrachromosomal nucleoli⁷⁻¹⁰. It has been reported that the premeiotic lampbrush chromosomes in these germinal vesicles are positively stained for actin and tubulin by the immunoperoxidase technique¹¹. Moreover, the longitudinal contraction of these chromosomes is ATP dependent¹². Therefore it has been suggested that actin participates in the structural organisation of the highly specialised lampbrush chromosomes. However, actin is not a major component of the metaphase chromosome scaffold¹³. The results reported here suggest that actin is involved in the condensation of *Xenopus* chromosomes.

We studied chromosome condensation and meiotic spindle formation in the presence of antibodies directed against actin and myosin in maturing oocytes of *Xenopus laevis*. Affinity purified human anti-actin antibodies (AAA), characterised elsewhere^{14,15}, and a γ -globulin fraction from rabbit serum containing antibodies against human uterine myosin (AMA)¹⁶ were injected into the cytoplasm or into the germinal vesicle of fully grown oocytes. As a control, we injected nonspecific human or rabbit γ -globulins. The injection technique has been described in detail elsewhere¹⁷. Progesterone was added to induce *in vitro* maturation¹⁸. The hormone treated oocytes

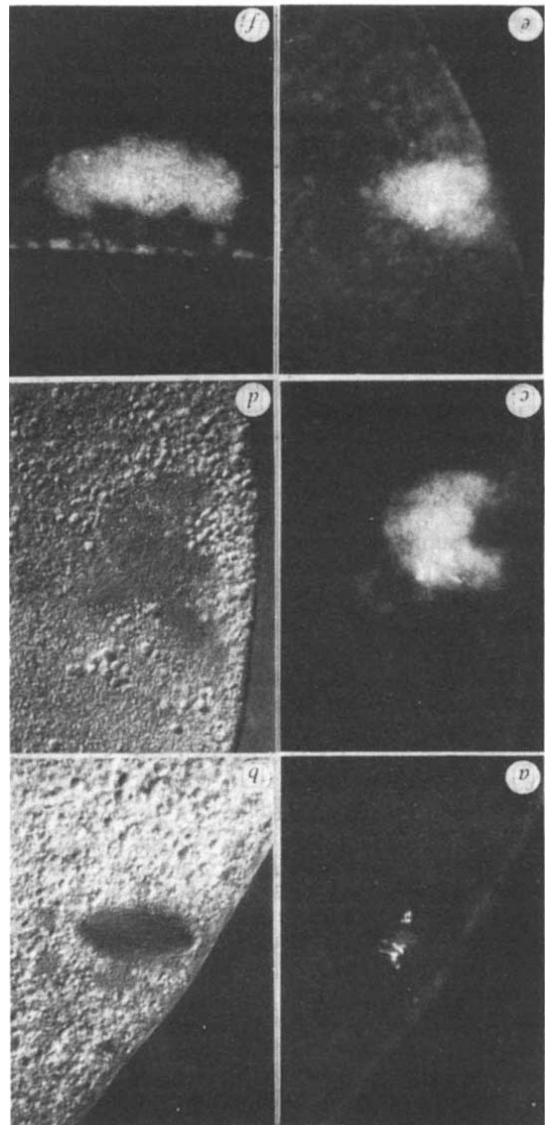


Fig. 1 Effect of AAA injection into the germinal vesicle of *Xenopus* oocyte on chromosome condensation and spindle formation. Fully grown *Xenopus laevis* oocytes were centrifuged¹⁹ (400g, 10 min, allowing nuclei to sink back into the cytoplasm after injection) and injected intranuclearly with 20 nl of injection buffer containing 0.4 mg per ml of either AAA or nonspecific human γ -globulins (Fluka) equivalent to 8 ng of proteins per nucleus. After 1 h, 10^{-5} M progesterone was added to induce meiosis. Oocytes showing a distinct white spot indicative of maturation were fixed in Smith's solution²⁰, dehydrated in butanol, embedded in paraffin, cut into 6- μ m thick serial sections, stained by the Feulgen technique and counterstained with light green. *a*, Fluorescence micrograph at wavelength 546 nm to demonstrate Feulgen-positive chromosomes in metaphase of an egg injected with nonspecific γ -globulins. Condensed chromosomes are normally organised on the spindle. *b*, Nomarski differential interference microscopy of the same field; the spindle appears normal. *c*, Fluorescence micrograph of Feulgen-positive material in a meiotic metaphase of an egg injected with AAA. Chromatin appears as a loosely arranged large spot with dense bodies scattered between. Note the positivity in the areas corresponding to asters. *d*, Nomarski differential interference microscopy of the same field as in (*c*); asters are present but the spindle is disorganised. *e*, and *f*, fluorescence microscopy of other examples of loose chromatin in meiotic metaphases of eggs after injection of AAA ($\times 700$).

undergo breakdown of the germinal vesicle and the first meiotic division, and then remain arrested in the second meiotic metaphase. The eggs were fixed, embedded in paraffin, cut

serially and stained by the Feulgen technique. Our experimental system, which thus facilitates cytological observation of chromosome condensation and spindle formation, is described in detail in the legend to Fig. 1.

Centrifuged oocytes injected with nonspecific human or rabbit γ globulins into either the cytoplasm or the nucleus produced normal meiotic spindles in three series of experiments. After nuclear injection of nonspecific γ -globulins, seven normal spindles were found in eight cases (Fig. 1a and b), the remaining egg having probably lost its spindle and chromatin through the injection hole—no Feulgen-positive material was found on any of the serial sections. In a few cases, we observed anaphase or telophase of the first meiotic division instead of second metaphase. This does not influence the observation of chromosome condensation because there is no despiralisation of chromosomes between the two meiotic divisions. These observations show that normal meiosis takes place in the oocytes after chemical defolliculation, centrifugation and nuclear injection of nonspecific human or rabbit γ globulins.

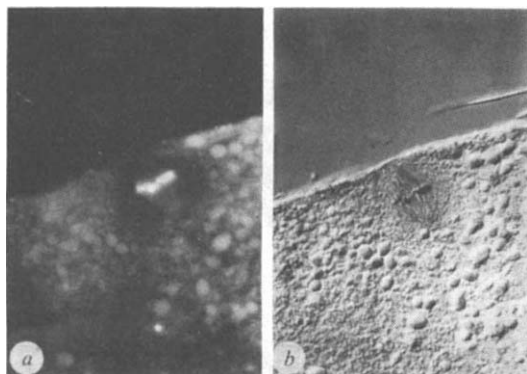


Fig. 2 Normal meiotic metaphase after injection of AMA into the nucleus of a *Xenopus* oocyte. Techniques are explained in the legend to Fig. 1. Nuclei were injected with 20 nl of injection buffer containing 2 mg per ml AMA γ -globulin fraction (DE 52 cellulose column) equivalent to 40 ng of proteins per nucleus. *a*, Fluorescence micrograph showing condensed Feulgen-positive chromosomes normally organised on the spindle. *b*, Nomarski differential interference microscopy of the same field; the spindle appears normal ($\times 700$).

Injection of AAA into the cytoplasm did not affect germinal vesicle breakdown, chromosome condensation or spindle formation, although three times more AAA was injected than into the nucleus. In all five cases, normally condensed chromosomes were observed, associated with normal spindles.

By contrast, the injection of AAA into the germinal vesicle of the oocyte severely affected chromosome condensation. In none of the 11 oocytes examined from three independent series were normal meiotic metaphase chromosomes formed. In all nine cases where chromatin was found in the sections, it was loosely arranged but in part associated with spindle asters. Figure 1c-f shows examples of the deficient chromosome condensation in the presence of intranuclear AAA. No effect on normal meiotic metaphase formation was observed after injection of rabbit γ -globulins containing AMA into the nucleus (Fig. 2).

These results show that in our experimental conditions, AAA, but not AMA, affected chromatin condensation when injected into the germinal vesicle of *Xenopus* oocytes. The lack of effect of AMA does not necessarily mean that myosin does not have a role in chromosome condensation. Alternatively, we could have used insufficient AMA, nuclear *Xenopus* myosin could be antigenically different from human uterine myosin, or the binding of AMA to myosin may not affect the activity of the protein in this respect. We do not know the mechanism of the inhibitory action of AAA on chromosome condensation. Since AAA are pre-

cipitating antibodies¹⁵, it is possible that they precipitate out the amount of protein that is instrumental in chromosome condensation.

After intracytoplasmic injection of AAA, metaphase spindles looked normal, perhaps because the nuclear envelope acted as a barrier—chromosome condensation probably starts before germinal vesicle breakdown. Moreover, large quantities of cytoplasmic actin could bind to the injected AAA and block their activity.

Actin and myosin have been described as constituents of the mitotic spindle²¹⁻²⁵. In our experiments, mitotic spindle formation was perturbed after intranuclear injection of AAA (Fig. 1d). However, asters were formed and small portions of the loose chromatin were found closely associated with the aster fibres (Fig. 1c). These experiments thus do not elucidate the possible role of actin in the attachment of chromosomes to spindle fibres and in spindle pole formation.

At any rate, our results suggest that actin is involved in chromosome condensation. Further work is needed to clarify whether actin exerts its influence by being an integral component of the prophase chromosome structure.

This work was supported by the Swiss National Science Foundation (grants no. 3.445.0.79 and 3.300.0.78).

Received 31 July; accepted 1 October 1979.

1. Jockusch, B. M., Becker, M., Hindennach, I. & Jockusch, H. *Expl Cell Res.* **89**, 241-246 (1974).
2. Douvas, A. S., Harrington, C. A. & Bonner, J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3902-3906 (1975).
3. Lestourgeon, W. M., Forer, A., Yang, Y.-Z., Bertram, J. S. & Rusch, H. P. *Biochim. biophys. Acta* **379**, 529-552 (1975).
4. Goldstein, L., Ko, C. & Erick, J. *Cell Biol. int. Rep.* **1**, 511-515 (1977).
5. Fukui, Y. *J. Cell Biol.* **76**, 146-157 (1978).
6. Rubin, R. W., Goldstein, L. & Ko, C. *J. Cell Biol.* **77**, 698-701 (1978).
7. Clark, T. G. & Merriam, R. W. *Cell* **12**, 883-891 (1977).
8. Clark, T. G. & Merriam, R. W. *J. Cell Biol.* **77**, 427-438 (1978).
9. Merriam, R. W. & Clark, T. G. *J. Cell Biol.* **77**, 439-447 (1978).
10. Clark, T. G. & Rosenbaum, J. *J. Cell Biol.* **79**, 268a (1978).
11. Karsenti, E., Gounon, P. & Bornens, H. *Biol. Cell* **31**, 219-224 (1978).
12. Karsenti, E. & Gounon, P. *Biol. Cell* **34**, 91-97 (1979).
13. Adolph, K. W., Cheng, S. W. & Laemmli, U. K. *Cell* **12**, 805-816 (1977).
14. Gabbiani, G. *et al. Am. J. Path.* **72**, 473-488 (1973).
15. Chaponnier, C., Kohler, L. & Gabbiani, G. *Clin. exp. Immun.* **27**, 278-284 (1977).
16. Gabbiani, G., Chaponnier, C. & Hüttner, I. *J. Cell Biol.* **76**, 561-568 (1978).
17. Runger, D. & Türlér, H. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6073-6077 (1978).
18. Schorderet-Slatkine, S. *Cell Differ.* **1**, 179-182 (1972).
19. Kressmann, A., Clarkson, S. G., Telford, S. L. & Birnstiel, M. L. *Cold Spring Harb. Symp. quant. Biol.* **42**, 1077-1082 (1977).
20. Smith, G. B. *J. Morph.* **23**, 61-153 (1912).
21. Aronson, J. F. *J. Cell Biol.* **26**, 293-298 (1965).
22. Sanger, J. W. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2451-2455 (1975).
23. Fujiwara, K. & Pollard, T. D. *J. Cell Biol.* **71**, 848-875 (1976).
24. Cande, W. Z., Lazarides, E. & McIntosh, J. R. *J. Cell Biol.* **72**, 552-567 (1977).
25. Mabuchi, I. & Okuno, M. *J. Cell Biol.* **74**, 251-263 (1977).
26. Sanger, J. W. in *Mitosis Facts and Questions* (eds Little, M. *et al.*) 98-113 (Springer, Berlin, 1977).

Mouse epidermal Ia molecules have a bone marrow origin

John G. Frelinger*, Leroy Hood*, Sharon Hill† & Jeffrey A. Frelinger†

* Division of Biology, California Institute of Technology, Pasadena, California 91125

† Department of Microbiology, University of Southern California Medical School, Los Angeles, California 90033

The major histocompatibility or H-2 complex of the mouse is divided into five regions (K, I, S, G and D)¹. Genes in the I region regulate immune responses². The I region has several subregions which are designated I-A, I-B, I-J, I-E and I-C. The I-A and I-E subregions also code for a set of serologically detected cell-surface alloantigens, designated Ia antigens³. The relationship between the genes regulating the immune

response and those encoding the serologically detectable alloantigens is still unknown. A number of species including man, rats and guinea pigs contain genetic regions apparently equivalent to the murine I region. Ia molecules are integral cell-surface glycoproteins that consist of two subunits of approximate molecular weights 35,000 (α) and 28,000 (β). Unlike the classical transplantation antigens which are present on almost all cells, the Ia antigens are found primarily on cells of the immune system—lymphocytes and macrophages⁴⁻⁶. A notable exception has been the demonstration of Ia antigens in mice, or Ia-like antigens in other mammals, on epidermal cells⁶⁻¹³. There is controversy about the numbers of Ia-positive cells in the epidermis. Fluorescence studies in humans^{11,12}, guinea pigs¹⁴ and mice¹⁵ indicate that only about 5% of epidermal cells are Ia positive. These cells were identified by morphological criteria as the macrophage-like Langerhans cells. However, cytotoxicity studies in mice using anti-Ia sera indicate that a majority of epidermal cells (up to 90%) are Ia positive⁶⁻⁸. The reason for this discrepancy is not known. Here we demonstrate that the epidermal Ia molecules are synthesised by bone marrow-derived cells, presumably Langerhans cells.

We replaced the entire population of bone marrow-derived cells in one animal with bone marrow cells from a second animal capable of synthesising Ia molecules distinct from the first. These chimaeric animals are constructed by the injection of donor bone marrow cells into lethally irradiated recipients. In chimaeric animals all cells except those of bone marrow origin are of the recipient genotype. We reasoned that if the Ia molecules synthesised in epidermal cell preparations (mouse tail cells) are synthesised by bone marrow-derived cells, the Ia molecules isolated from long-term (>3 months) chimaeras should be of donor origin. Alternatively, if the Ia molecules are synthesised by a majority of epidermal cells, the Ia molecules should be of recipient origin.

Table 1 Source of haematopoietic cells in chimaeric mice

Target	Anti-K ^b H-2.33*	Anti-D ^b H-2.2†	Anti-K ^k H-2.23*	Anti-D ^d H-2.4†
Donor→Recipient				
IR→(B10×1R)F ₁	<10 (<10)	NT	320 (>90)	NT
(B6×A)F ₁ →B6	NT	80	NT	>320
Controls				
1R	<10 (<10)	160	320 (>90)	<10
B10	>640 (>80)	160	<10 (<10)	<10
A	<10 (<10)	<10	320 (>90)	>320
(B6×A)F ₁	NT	80	NT	>320
(B10×1R)F ₁	>640 (>90)	NT	320 (>90)	NT

Chimaeras were produced as described by Von Boehmer²⁰. Mice were irradiated with 925 rads from a linear accelerator and reconstituted with $1-2 \times 10^7$ anti-mouse brain ($\alpha\theta$) plus complement-treated bone marrow cells. Mice were tested for chimaerism after 3 months. NT, not tested.

*Tested by dye exclusion microcytotoxicity on peripheral blood leukocytes. Numbers represent reciprocal of the antiserum dilution giving 50% killing of targets and in parentheses, the maximum attainable killing. Sera for cytotoxicity: anti-H-2.33 (A×B10.D2)F₁ anti-B10.A(5R); anti-H-2.23, (B10×A.TL)F₁ anti-A.AL.

†Tested by polyvinylpyrrolidone (PVP) haemagglutination of red blood cells. Number represents the reciprocal of the greatest antiserum dilution which permits detectable agglutination reaction. Sera for haemagglutination: anti-H-2.2, (A×B10.D2)F₁ anti-B10.A(2R); anti-H-2.4, (B10.A(1R)×A.SW)F₁ anti-B10.A.

Our experimental design requires almost complete replacement of recipient bone marrow-derived cells with donor cells. Direct tests confirmed that this had occurred (Table 1). Two types of chimaeric animal were constructed: parental (P) cells were transplanted to F₁ offspring (P→F₁) and vice versa (F₁→P). For the P→F₁ chimaeras, the analysis of bone marrow-derived cells used cytotoxicity and haemagglutination assays. In the case of the F₁→P chimaeras, the donor cells in the recipient were measured by haemagglutination. In both cases, the results

indicated that the haematopoietic system was almost completely reconstituted with donor cells (Table 1).

We isolated epidermal Ia molecules by indirect immunoprecipitation using mouse alloantisera as previously described¹⁰. The specificity of the antisera is critical in determining whether the Ia is contributed by donor or recipient cells in these chimaeras. The sera were characterised extensively by cytotoxicity, absorption and immunoprecipitation (data not shown). They were tested on strains identical to those used in these experiments to demonstrate unequivocally that the observed reaction was specific for recipient or donor strains. An F₁ animal

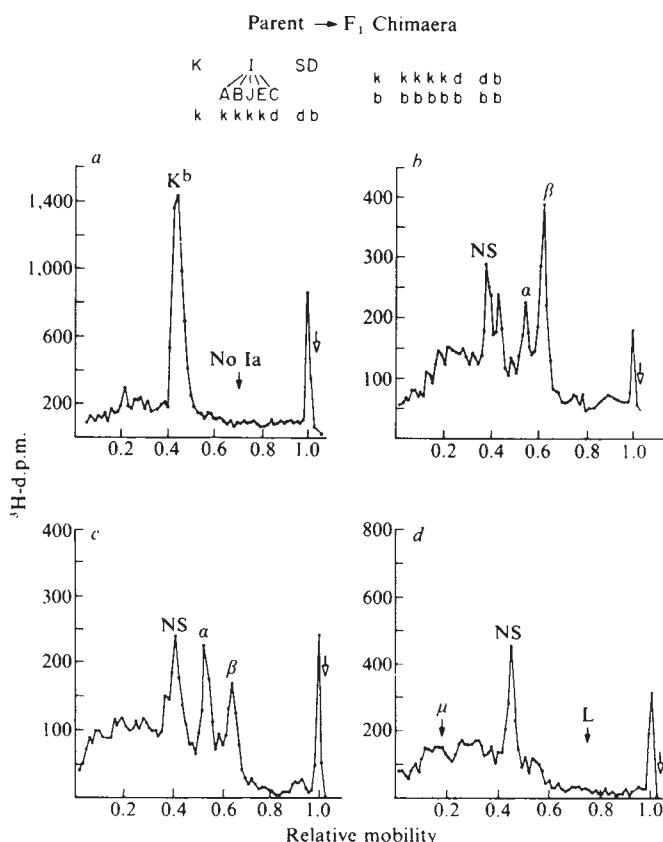


Fig. 1 SDS-polyacrylamide gel electrophoresis of epidermal Ia molecules isolated from parent→F₁ bone marrow chimaeras. Epidermal Ia was isolated as previously described¹⁰. Briefly, single cell suspensions of trypsinised epidermal cells were biosynthetically labelled with ³H-amino acids and lysed in 0.5% NP40. The glycoprotein fraction was isolated by *Lens culinaris* lectin affinity chromatography, concentrated fivefold in a B-15 Minicon, and indirectly immunoprecipitated with specific antisera and fixed *Staphylococcus aureus* (Cowan I strain). The parent→F₁ chimaera extracts were precipitated with (A×B10.D2)F₁ α B10.A(5R) [αK^bI-A^bI-B^b] (a) (B10×HTT)F₁ α B10.A(5R) [αI-E^dI-C^k] (b) (A.TH×B10.HTT)F₁ α A.TL[αI-A^kI-B^kI-J^k] (c) rabbit anti-MOPC 104E [Rαμ] (d). ↓ Arrows indicate MOPC 104E heavy chain (μ) and light chain markers; | arrows indicate the dye front. NS represents nonspecific 45K peaks, possibly actin.

[B10.A(1R)×B10] is heterozygous for the H-2 complex and expresses Ia molecules encoded by both the H-2^b and H-2^d haplotypes (Fig. 1). In F₁ chimaeras (P→F₁), only Ia molecules from the donor animal can be isolated from epidermal cells. Both I-A and I-E subregion products can be detected (Fig. 1b, c). In these chimaeras, we cannot detect Ia molecules of the H-2^b haplotype from the B10 parent (Fig. 1a). In contrast, transplantation antigens of both haplotypes can readily be detected in these chimaeric animals. The results in Fig. 1a also provide direct evidence that transplantation molecules (K^b) can be synthesised by a non-bone marrow-derived cell. We should stress that immunoprecipitation with a rabbit anti-μ serum

showed no evidence of contaminating IgM-positive cells in the epidermal cell population (Fig. 1d). Thus, the donor Ia must be derived from a population of bone marrow cells distinct from B cells.

In the chimaera described above, we could only demonstrate a loss of one haplotype of Ia antigens from tail cell preparations derived from $P \rightarrow F_1$ chimaeras. To demonstrate directly the appearance of new Ia antigens not present in the host, $F_1 \rightarrow P$ chimaeras were constructed. The $[B6 \times A]F_1$ animal has Ia molecules of the H-2^b (B6) and H-2^a (A) haplotypes. In $[B6 \times A]F_1 \rightarrow B6$ chimaeras, we could detect the presence of Ia molecules of both the H-2^a and H-2^b haplotypes by immunoprecipitation. I-A Subregion products from both H-2^b and H-2^a haplotypes were easily detected (Fig. 2a, b). The A^k molecules can only be derived from the bone marrow cells. Both A^k and E^k molecules are present in these chimaeras (E^k not shown). Thus, new epidermal Ia antigens are derived from the donor bone marrow cells. These data from the $P \rightarrow F_1$ and $F_1 \rightarrow P$ chimaeras strongly suggest that the Ia molecules from chimaeric epidermal cells originate from bone marrow-derived cells.

The original reports of Ia molecules on mouse epidermal cells were based on adsorption analysis and antibody-mediated cytotoxicity using dye exclusion⁶. Other investigators independently confirmed these results^{7,8}. Using an indirect immunofluorescence assay, investigators working with guinea pigs, man and mouse, have suggested that the Ia is confined to a small subpopulation of epidermal cells, the macrophage-like Langerhans cells^{11,12,14,15}. In guinea pigs, biosynthetically labelled Ia molecules can only be immunoprecipitated from a Langerhans cell-enriched fraction, and not from a Langerhans cell-depleted fraction of epidermal cells¹⁶. Our experiments suggest that the biosynthetically labelled Ia molecules are derived from a cell of bone marrow origin. These observations suggest that a small number of cells in the epidermis are derived from the bone marrow and express Ia molecules. Although we have no direct evidence for the bone marrow origin of Langerhans cells, the fluorescence studies in guinea pigs, man and mouse¹⁵, together with the present results, suggest that the Langerhans cell produces the epidermal Ia molecules and has a bone marrow origin. This possibility is not surprising considering the macrophage-like functions ascribed to the Langerhans cells^{17,18}. Macrophages are generally thought to be derived from bone marrow precursors. However, our experiments detect only newly synthesised Ia molecules. The sensitivity of detection is a function of both the number of Ia molecules and their turnover rate. We are aware that Ia molecules could be synthesised by non-bone marrow-derived cells, and not detected in our experiments, if the Ia molecules were in much lower concentration per cell or turning over more slowly.

Differential sensitivity of immunofluorescence and cytotoxicity assays, or bystander effects in the cytotoxic assays or contaminating Sk antibody might explain the conflicting cytotoxicity⁶⁻⁸ and immunofluorescence^{11,12,14,15} data. At present, we feel the simplest interpretation of our data is that the Ia molecules present in the epidermis are synthesised by bone marrow-derived cells, probably the Langerhans cells.

Phagocytic macrophage cells are involved in the presentation of antigen to T cells. The subsequent activation and proliferation of the T cells involves the Ia molecules on the antigen-presenting macrophage¹⁹. In guinea pigs, Langerhans cells can function both as stimulators in a mixed lymphocyte reaction and as antigen-presenting cells¹⁶. Perhaps Langerhans cells in the epidermis function like macrophages¹⁸. A high concentration of antigen-presenting cells in the skin is reasonable, as the skin is the first line of defence against pathogens. It was suggested that non-lymphoid Ia-positive epithelial tissues also might be immunologically relevant¹³. The precise immunological implications of Ia molecules on cells in the epidermis are unknown. However, the observations reported here are intriguing because they clearly raise the possibility that Ia molecules may be restricted to cells involved in the immune response.

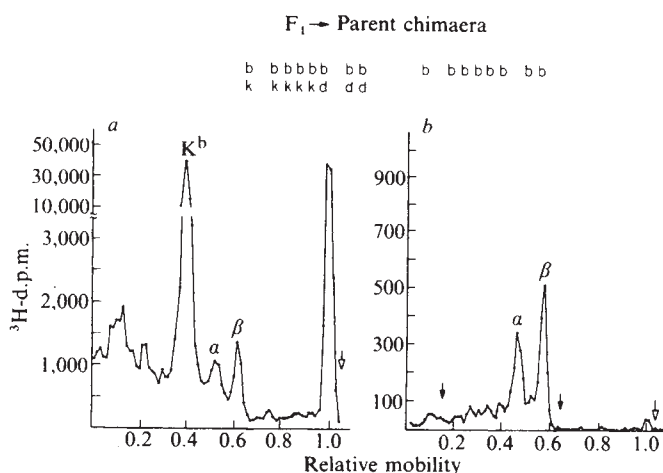


Fig. 2 SDS-polyacrylamide gel electrophoresis of epidermal Ia molecules isolated from $F_1 \rightarrow$ parent bone marrow chimaeras. The $F_1 \rightarrow$ parent chimaera extracts were precipitated with $(A \times B10.D2)F_1 \alpha B10.A(5R) [\alpha K^b I-A^b I-B^b]$ (a) and CWB α C3H hybridoma 10-2.15 $[\alpha-A^k]$ (b). This reagent has been characterised to react with A^k (ref. 21). We tested the hybridoma reagent using a two-stage dye exclusion cytotoxicity assay. No killing of B10 spleen cell targets was seen with this reagent (cytotoxic titre <1/10). A/Sn spleen cell targets were efficiently lysed (titre of >1/640 with 50% maximum cytotoxicity). In tail skin preparations from chimaeras, the ratio of transplantation antigens (K^b) to Ia molecules is altered compared with normal tail skin preparations¹⁰ (a). In chimaeras, there are fewer Ia molecules relative to transplantation antigens. The reason for this altered ratio is not clear. It seems likely that it represents the partial repopulation of epidermal Ia-bearing cells from bone marrow precursors. The monoclonal antibody used in b always gives smaller nonspecific peaks than immunoprecipitations using alloantisera. See Fig. 1 legend for explanation of markers.

We thank Glen Matsushima for technical assistance in antiserum production, Marilyn Kehry for donating the rabbit anti-MOPC 104E (R $\alpha\mu$), Dr Len Herzenberg for providing the anti-I-A^k-producing hybridoma through the Salk Institute, and Mitch Kronenberg for helpful discussions. J.G.F. is supported by a National Research Service Award (5 T32 GM07616), and J.A.F. is the recipient of American Cancer Society Faculty Research Award FRA-179. This research is supported by NIH grants AI 10781 and CA 22662.

Received 26 April; accepted 7 September 1979.

- Klein, J. *Biology of the Mouse Histocompatibility-2 Complex*, 389-489 (Springer, New York, 1975).
- Shreffler, D. C. & David, C. S. *Adv. Immun.* **20**, 125-195 (1975).
- Murphy, D. B., Herzenberg, L. A., Okumura, K., Herzenberg, L. A. & McDevitt, H. O. *J. exp. Med.* **144**, 699-712 (1976).
- Hauptfeld, V., Hauptfeld, M. & Klein, J. *J. Immun.* **113**, 181-188 (1974).
- Colombani, J., Colombani, M., Shreffler, D. C. & David, C. *Tissue Antigens* **7**, 74-85 (1976).
- Hammerling, G. J., Mauve, G., Goldberg, E. & McDevitt, H. O. *Immunogenetics* **1**, 428-437 (1975).
- Klein, J., Geib, R., Chiang, C. & Hauptfeld, V. *J. exp. Med.* **143**, 1439-1452 (1976).
- Krcso, C. J., Steinmuller, D. & David, C. S. *Cell. Immun.* **46**, 239-246 (1979).
- Delovitch, T. & McDevitt, H. O. *Immunogenetics* **2**, 39-52 (1975).
- Frelinger, J. G., Wettstein, P. J., Frelinger, J. A. & Hood, L. *Immunogenetics* **6**, 125-135 (1978).
- Klareskog, L., Tjerlund, U. M., Forsum, U. & Peterson, P. A. *Nature* **268**, 248-250 (1977).
- Rowden, G., Lewis, M. G. & Sullivan, A. K. *Nature* **268**, 247-248 (1977).
- Winman, K. *et al. Nature* **276**, 711-713 (1977).
- Stingl, G., Katz, S. I., Shevach, E. M., Wolff-Schreiner, E. & Green, I. *J. Immun.* **120**, 570-578 (1978).
- Rowden, G., Phillips, T. M. & Delovitch, T. L. *Immunogenetics* **7**, 465-478 (1978).
- Stingl, G., Katz, S. I., Clement, L., Green, I. & Shevach, E. M. *J. Immun.* **121**, 2005-2013 (1978).
- Prunieras, M. *J. invest. Derm.* **52**, 1-17 (1969).
- Silderberg-Sinakin, I., Thorbecke, G. J., Baer, R. L., Rosenthal, S. A. & Berezonsky, V. *Cell. Immun.* **25**, 137-151 (1976).
- Schwartz, R. A., Yano, A., Stimpfling, J. H. & Paul, W. E. *J. exp. Med.* **149**, 40-57 (1979).
- Oi, V. T., Jones, P. P., Goding, J. W., Herzenberg, L. A. & Herzenberg, L. A. in *Current Topics in Microbiology and Immunology* Vol. V, 81 (Springer, New York, 1978).
- Von Boehmer, H., Sprent, J. & Nabholz, M. *J. exp. Med.* **141**, 322-334 (1975).

Epidermal Langerhans cells are derived from cells originating in bone marrow

Stephen I. Katz, Kunihiro Tamaki & David H. Sachs*

Dermatology and *Immunology Branches, National Cancer Institute, Bethesda, Maryland 20205

Langerhans cells constitute a morphologically well characterised subpopulation (3–8%) of mammalian epidermal cells which, in contrast to the bulk of epidermal cells, bear Fc-IgG and C3 receptors¹, express immune response-associated (Ia) antigens and function as antigen-presenting cells and allogeneic stimulatory cells to primed T lymphocytes^{2–5}. The ontogeny of Langerhans cells has been a subject of considerable debate since their discovery⁶. Although some studies suggest that Langerhans cells are of mesenchymal as opposed to neural or melanocytic origin⁷, direct evidence for this has not been presented. In this study we demonstrate that, after 3 weeks, most of the Langerhans cells (LC) in parental skin which had been transplanted on to F₁ hybrids were of recipient origin whereas keratinocytes remained of donor origin; this indicates that the LC are derived from a mobile pool of cells. Furthermore, in studies of skin from radiation-induced bone marrow chimaeric animals we found that, depending on the strain combination, up to 80% of the epidermal LC were derived from the bone marrow of the donor animals.

Previous studies have demonstrated that in mice^{8,9} as well as in guinea pigs and humans, H-2 (B region in guinea pigs and HLA-A or -B in humans) antigens are expressed on the great majority of epidermal cells. In contrast, Ia antigens or their equivalents in mice, guinea pigs and humans are detected only on epidermal Langerhans cells^{1–5,8,9}. Langerhans cells can also be identified by light microscopy when they are incubated with IgG-coated ox red blood cells (EA-IgG), as they form rosettes through their Fc receptors. Our studies used epidermal cell suspensions prepared by trypsinisation (1% for 60 min at 37 °C), and epidermal cell-surface characteristics were identified by using a simultaneous rosette and indirect immunofluorescence (IF) assay⁹.

First, we transplanted sex-matched parental skin from A/J mice on to CAF₁ (BALB/c × A/J) hybrid mice and, after

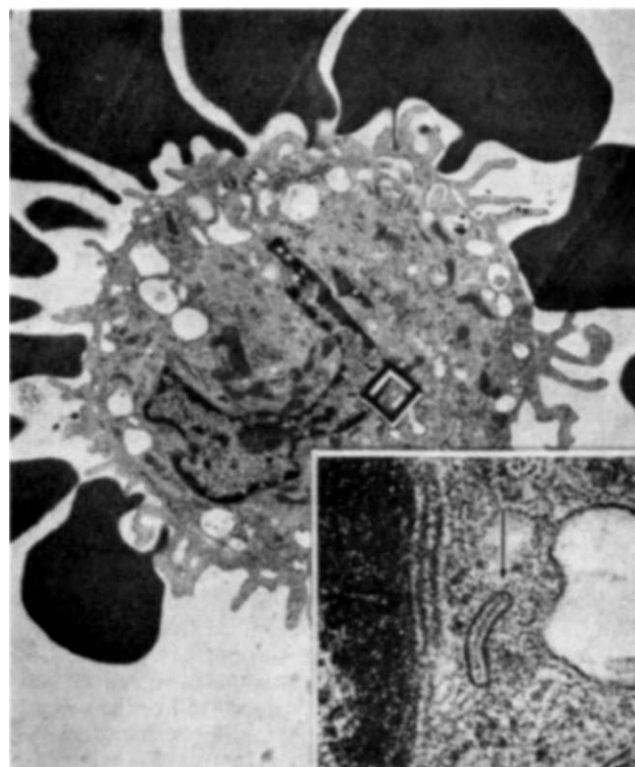


Fig. 1 Electron micrograph of an Fc-IgG-rosette-forming Langerhans cell which exhibits a lobulated nucleus, dendritic projections and typical Langerhans cell granule ($\times 4,000$, inset $\times 63,000$).

various time periods, determined whether the H-2 and Ia antigens of the LC and other epidermal cells were those of the donor or recipient. Table 1 shows that using an anti-Ia.8 antiserum which detects the Ia of BALB/c but not of A/J mice, 3.5–4% of the epidermal cells of the A/J transplants bore Ia antigens characteristic of the CAF₁ mice. The great majority of these were LC, as from 61% (11 d post-transplantation) to 100% (49 d post-transplantation) of the Fc-IgG-rosetting cells (Langerhans cells) were positive with the anti-Ia.8 reagent. Using an anti-H-2^d antiserum [(B10 × A)F₁ anti-B10.D2] we found that most of the epidermal cells were not of CAF₁ origin. Furthermore when syngeneic transplants (A/J skin on to A/J mice) were carried out, the anti-Ia.8 antiserum did not stain any of the A/J epidermal cells whereas 4.2% of these epidermal

Table 1 Detection of major histocompatibility complex alloantigens on transplanted skin

Donor	Recipient	Day tested	Serum 1 (anti-Ia ^d)		Antisera Serum 2 (anti-Ia ^d , H-2 ^d)		Serum 3 (anti-Ia ^{k,d})	
			% IF +ve*	% Rosette +ve†	% If +ve	% Rosette +ve	% IF +ve	% Rosette +ve
A/J	A/J	14	0	0	0	0	4.2	95
	CAF ₁ (A/J × BALB/c)		5	95	76	92	4.2	100
A/J	CAF ₁	11	4‡	61	6.9	74	NT	NT
		14	3.5	61	8	60	NT	NT
		21	3.5	95	9	100	5	95
		49	3.8	100	8	100	5	90

The antisera used have been extensively characterised by cytotoxicity and absorption analyses on spleen and lymph node cells. Serum 1 is a (B10.A × A)F₁ anti-B10 which is negative on H-2^a(A/J) and when tested on H-2^d(BALB/c) reacts only with the I-A specificity Ia.8. Serum 2 is a (B10 × A)F₁ anti-B10.D2 which is also negative on H-2^a and when tested on BALB/c reacts with the H-2K specificities H-2.31 and the Ia specificities Ia.8 and 11. Serum 3 is an A.TH anti-A.TL which is a polyvalent anti-Ia antiserum, and reacts with multiple Ia specificities of both H-2^a and H-2^d. NT, Not tested.

* Per cent of viable cells which fluoresce.

† Per cent of Fc-IgG-receptor-positive cells (Langerhans cells) which fluoresce.

‡ Each result represents the mean of two or three experiments.

Table 2 Detection of major histocompatibility alloantigens on epidermal cells of chimaeric mice

Donor	Recipient	Day tested	Antisera					
			Serum 1 (anti-H-2 ^d , Ia ^d)		Serum 2 (anti-H-2 ^b , Ia ^b)		Serum 3 (anti-Ia ^d)	
			% IF +ve*	% Rosette +ve†	% IF +ve	% Rosette +ve	% IF +ve	% Rosette +ve
BALB/c	C57BL/6	48	2.7‡	20	68	88	NT	NT
		62	2.9	45	70	58	NT	NT
		89	1.5	30	81	83	2.4	27
Controls								
	BALB/c(H-2 ^d , Ia ^d)		83	91	0	0	2.9	80
	C57BL/6(H-2 ^b , Ia ^b)		0	0	78	97	0	0

The antisera used have been extensively characterised by cytotoxicity and absorption analysis on spleen and lymph node cells. Serum 1 is a (B10 × A)F₁ anti-B10.D2 which is negative on H-2^b and when tested with BALB/c reacts with the H-2K specificities H-2.31 and the Ia specificities Ia.8 and 11. Serum 2 is a B10.D2 anti-B10 which is negative on H-2^d and reacts with H-2^b and Ia^b of the C57BL/6 mice. Serum 3 is a (B10.A(4R) × A.BY)F₁ anti-B10.A(2R) which is negative on H-2^b and reacts with Ia^d of BALB/c. NT, Not tested.

* Per cent of viable cells which fluoresce.

† Per cent of Fc-IgG-receptor-positive cells (Langerhans cells) which fluoresce.

‡ Each result represents the mean of two or three experiments.

cells were IF positive with a polyvalent anti-Ia antiserum (A.TH anti-A.TL) detecting Ia antigens of H-2^a origin. These findings suggest that the Langerhans cells of the transplanted skin were derived from a mobile pool of recipient cells.

Our second set of experiments was designed to assess more directly the origin of the Langerhans cells. Chimaeric animals were prepared by irradiating mice with 750–900 R and reconstituting with 2.5–3 × 10⁷ allogeneic or semi-allogeneic bone marrow cells. All mice used in our studies were shown to be 'totally' chimaeric by the demonstration of H-2 and Ia antigens of the donor strain on spleen and peripheral blood cells. Table 2 demonstrates that after various periods of time following chimaerisation (BALB/c into C57BL/6) 20–45% of the Fc-IgG-bearing cells were of donor origin whereas the remainder of the Fc-IgG-bearing cells were of recipient origin. These studies also show that the bulk of the epidermal cells (keratinocytes) come from the recipient animal, as most of the epidermal cells were IF positive with the anti H-2^b (B10.D2 anti-B10) antiserum. In another strain combination (B10 × B10.A)F₁ into B10.A a much larger percentage of Fc-IgG-bearing cells were found to be of donor origin 80–85 d after chimaerisation (Table 3). Here we determined that 68% [using a (B10.A × A)F₁ anti-B10.D2 antiserum] to 80% [using a (B10.A × A)F₁ anti-B10 antiserum] of the Fc-IgG-rosetted cells were of donor bone marrow origin. Electron microscopic studies of the Fc-IgG-bearing rosetted cells demonstrated that most of the cells had all the characteristics of Langerhans cells, including the unique cytoplasmic organelles (Fig. 1).

Taken together, these studies indicate that epidermal Langerhans cells are derived from and are continually re-

populated by a mobile pool of precursor cells which for the most part originate in the bone marrow. The most likely precursor cell is the monocyte, as Langerhans cells exhibit similar, if not identical, surface marker characteristics to cells of the monocyte-macrophage series. None of our chimaera studies produced total replacement of epidermal LC by donor precursor cells. This may be due to the fact that residual host precursor monocytes remain in the bone marrow at the dose of irradiation used, even though the peripheral lymphoid cells are completely replaced. This possibility is under study. The demonstration of the non-total replacement of the monocyte pool may be important for the interpretation of mechanistic studies using allogeneic chimaeras.

Langerhans cells probably subserve functions of the Ia-rich relatively non-phagocytic macrophages and may not only function in an antigen-presenting capacity for exogenous allergens but also represent the 'passenger leukocyte' which is thought to induce graft rejection. In this regard, Steinmuller has reported sensitisation of animals by transplantation of skin from chimaeras¹⁰. In these experiments, skin was transplanted from chimaeras (A recipient, (A × B)F₁ donor) on to strain A mice. The grafts were accepted, but when skin was then transplanted from B animals on to the same A mice, the second grafts were rejected in an accelerated fashion. The most likely source of the B antigens in the chimaera skin was the haematopoietically derived cells from the donor. Our data indicate that the chimaeric skin becomes rich in donor Ia because of the appearance of donor Langerhans cells in the epidermis. It is likely that these cells have an important role in allogeneic graft rejection. Such conclusions are consistent with our previous studies

Table 3 Detection of major histocompatibility alloantigens on epidermal cells of chimaeric mice

Donor	Recipient	Antisera							
		Serum 1 (anti-H-2 ^b)		Serum 2 (anti-H-2 ^a)		Serum 3 (anti-Ia ^b)		Serum 4 (anti-Ia ^a)	
		% IF +ve*	% Rosette +ve†	% IF +ve	% Rosette +ve	% IF +ve	% Rosette +ve	% IF +ve	% Rosette +ve
(B10 × B10.A)F ₁ (H-2 ^{a/b} , I ^{a/b})	B10.A(H-2 ^a , Ia ^a)	6.3‡	80	91	81	4.5	68	5.1	78
Controls									
A/J(H-2 ^a , Ia.7)		0	0	95	100	0	0	3.8	100
C57BL/6(H-2 ^b , Ia.8)		75	96	0	0	3.6	75	0	0

The animals were tested 80–85 d after chimaerisation. The antisera used have been extensively characterised by cytotoxicity and absorption analysis on spleen and lymph node cells. Serum 1 is a (B10.A × A)F₁ anti-B10 which is negative on H-2^a and reacts with H-2^b and Ia^b. Serum 2 is an A.BY anti-A/J which is negative on H-2^b and reacts with H-2^a. Serum 3 is a (B10.A × A)F₁ anti-B10.D2 which is negative on H-2^a and reacts with the Ia^b specificity Ia.8. Serum 4 is an (A.TH × B10.S)F₁ anti-B10.HTT and is negative on H-2^b and reacts with the Ia^a specificity Ia.7.

* Per cent of viable cells which fluoresce.

† Per cent of Fc-IgG-receptor-positive cells (Langerhans cells) which fluoresce.

‡ Each result represents the mean of two experiments.

demonstrating the antigen-presenting and allogeneic-stimulatory capacities of Langerhans cells⁵.

The chimaeric animals were provided by Drs R. Almarez and A. Singer.

Received 7 June; accepted 31 August 1979.

1. Stingl, G. *et al.* *Nature* **268**, 245–246 (1977).
2. Rowden, G., Lewis, M. G. & Sullivan, A. K. *Nature* **268**, 247–268 (1977).
3. Klareskog, L., Malmnas-Tjernlund, U., Forum, U. & Peterson, P. A. *Nature* **268**, 248–250 (1977).
4. Stingl, G., Katz, S. I., Shevach, E. M., Wolff-Schreiner, E. Ch. & Green, I. *J. Immun.* **120**, 570–578 (1978).
5. Stingl, G., Katz, S. I., Clement, L., Green, I. & Shevach, E. M. *J. Immun.* **121**, 2005–2013 (1978).
6. Langerhans, P. *Virchows Arch. path. Anat. Physiol.* **44**, 325–337 (1968).
7. Breathnach, A. S., Silvers, W. K., Smith, J. & Heyners, S. J. *Invest. Derm.* **50**, 147–150 (1968).
8. Rowden, G., Phillips, T. M. & Delovitch, T. L. *Immunogenetics* **7**, 465–478 (1978).
9. Tamaki, K., Stingl, G., Gullino, M., Sachs, D. H. & Katz, S. I. *J. Immun.* **123**, 784–787 (1979).
10. Steinmuller, D. *Transplant Proc.* **1**, 593–596 (1969).

Corneal allografts fail to express Ia antigens

J. Wayne Streilein, Galen B. Toews
& Paul R. Bergstresser

Departments of Cell Biology and Internal Medicine,
University of Texas Health Science Center at Dallas,
5323 Harry Hines Blvd, Dallas, Texas 75235

Although the surface determinants encoded by class I genes of the major histocompatibility complex (murine K/D; human HLA-A/B/C) are expressed on virtually all nucleated cells, the molecular products of class II genes (murine I, human HLA-D/DR) are expressed only on B lymphocytes, macrophages, sperm and certain subsets of T lymphocytes, especially suppressor cells^{1,2}. Controversy exists concerning the expression of class II antigens on cells of the epidermis. On the one hand, Hammerling *et al.*³ and Frelinger *et al.*⁴ have used serological methods to detect Ia antigens on epidermal cells. On the other hand, fluorescein isothiocyanate-conjugated antisera have been used to show that human and guinea pig class II alloantigens are only expressed on Langerhans cells in the epidermis^{5–7}. Recently, Rowden *et al.*⁸ have convincingly demonstrated that Ia antigens are expressed exclusively on Langerhans cells within murine epidermis. We have now shown that cornea, which is an epidermal tissue devoid of Langerhans cells, does not express Ia antigens.

Langerhans cells have been found to be closely associated with the induction and expression of delayed cutaneous hypersensitivity reactions elicited by chemical contactants^{9,10}, but not all cutaneous sites are equally endowed with these cells, the density of which varies considerably in skin from mouse ears, foot pad and body wall¹¹. Similarly, we have recently demonstrated marked variation in Langerhans cell-surface density using an ATPase staining method in mice¹². The most dramatic difference from body wall skin, which contains approximately 800 ± 200 Langerhans cells per mm², was found in the cornea

which is completely free of ATPase-positive, dendritic cells, that is, Langerhans cells. This observation is consistent with a previous series of studies^{12–15}. Recently, it has been claimed that Langerhans cells, as defined by surface Ia antigens, are present at the periphery of the cornea and the limbus¹⁶. We also find ATPase-positive cells at the corneal limbus. In the grafting experiments described below, we took special care to prepare corneal grafts by severely cutting away peripheral tissues, including the limbus.

To determine whether alloantigens encoded by the I region of the murine H-2 complex can be expressed functionally in epidermal tissue if Langerhans cells are not present we placed corneal grafts on prepared beds on thoracic walls of recipient mice that differed from their corneal graft donors at the K region alone, at combined K and I regions, and at the I region alone. These corneal allografts were observed for evidence of rejection over a 45-d interval. Recipients were then rechallenged with conventional body wall skin grafts syngeneic to the original corneal allografts. Median survival times (MSTs) were calculated and compared with appropriate controls to determine whether first- or second-set patterns of rejection had taken place.

The fate of syngeneic corneal grafts must first be described. All grafts were carefully trimmed of scleral and limbic tissues and placed, endothelial-side down, on the raw sub-dermal surface. Each graft bed received three corneal grafts. The site was covered with Vaseline-impregnated gauze and wrapped with plaster of Paris bandage. Plaster casts were first removed for graft inspection 8 d later. At that time, three circular, transparent corneal grafts were usually found. When one of the grafts had been placed strategically over a subcutaneous blood vessel of significant size, blood could be seen coursing through the vessel directly beneath the transparent graft. Corneal grafts retained their transparency so long as they were covered and dressed with the Vaseline gauze. After exposure to room air, corneal transparency gradually gave way over several days to translucency which then persisted indefinitely. Thereafter, corneal grafts were identifiable by their smooth surface, and by the slightly dome-shaped contour due to the inherent structure of the corneal stroma. Healthy autografts of this type have been observed beyond 60 d. There was very little wound contracture at these graft sites during the observation period.

The results of the allografting experiments described in outline above are presented in Table 1. H-2-congenic strains B10.A and B10.AQR differ at the K region: B10.A possesses the K^k allele, whereas B10.AQR possesses the K^q allele. The MST of first-set B10.A body wall skin grafts placed in B10.AQR recipients was 16 ± 1.1 d. The MST of B10.A corneal allografts on B10.AQR recipients was similar, 13 ± 1.1 d. During their early course the gross appearance of these corneal allografts was quite similar to that of syngeneic corneal grafts. However, 1–2 d before rejection, the graft beds became acutely inflamed; the surface epidermis of the grafts developed hyperkeratotic changes and then quickly sloughed, to be replaced by a dried eschar. Within the next 24–48 h, wound contracture began and by 5 d, only a small, crusted wound, a few millimetres in diameter, remained.

Table 1 Comparative sensitising capacities of allografts prepared from cornea and body wall skin

Recipient	First-set graft					Second-set graft				
	Donor	H-2 Disp.	Graft source	n	MST	Donor	H-2 Disp.	n	MST	
1. B10.AQR	B10.A	K ^k	Body wall	13	16.0 ± 1.1	B10.A	K ^k	13	9.0 ± 1.1	
2. B10.AQR	B10.A	K ^k	Cornea	11	13 ± 1.1	B10.A	K ^k	11	9.0 ± 1.2	
3. A.TH	A.TL	I ^k	Body wall	10	16 ± 1.3	A.AL	K ^k I ^k	10	9.5 ± 1.3	
4. A.TH	A.AL	K ^k I ^k	Body wall	10	14.8 ± 1.1	A.AL	K ^k I ^k	10	9.0 ± 1.0	
5. A.TH	A.TL	I ^k	Cornea	13	>45	A.AL	K ^k I ^k	13	14.8 ± 1.1	
6. A.TH	None					A.AL	K ^k I ^k	8	15.0 ± 1.1	

All second-set grafts were prepared from body wall skin. H-2 disp. refers to H-2 differences between donor and recipient. I = ABIEC subregions plus the S and G regions of H-2. MST = Median survival time in days, ± 1 s.d.

H-2-congenic strains A.TL and A.TH differ from each other across the entire I region of H-2, plus the S and G regions: A.TL expresses the I-ABJCSG alleles of k, whereas A.TH expresses comparable alleles of the s haplotype. First-set body wall skin grafts from A.TL on A.TH recipients were rejected acutely, MST 16 ± 1.3 d (Table 1, line 3). In contrast, A.TL corneal allografts survived on A.TH recipients beyond 45 d (line 5). No grafts were rejected. In fact, the appearance of these grafts throughout the observation interval was virtually identical to that of syngeneic corneal grafts. These data indicate that epidermis deficient in Langerhans cells is similarly deficient in expression of alloantigenic products of I-region genes and support the claim that only Langerhans cells within the epidermis express I-region determinants in an immunogenically important way.

This interpretation was strengthened by the findings of the next experiments. Recipients of body wall and corneal first-set allografts were regrafted with body wall skin prepared from donors expressing the alloantigens putatively present on the first-set grafts. Thus, when B10.AQR animals were rechallenged with B10.A body wall skin grafts, they rejected these grafts in an accelerated, second-set manner, indicating that body wall skin and cornea are equally able to sensitise to the alloantigens of the K region (Table 1, lines 1, 2). Similarly, A.TH animals grafted first with A.TL body wall skin rejected test grafts of A.AL skin bearing identical I^k determinants in a second-set fashion (MST of 9.5 compared with 16 d, Table 1, line 3). However, when A.TH recipients of I-region-disparate A.TL corneal grafts were challenged with body wall skin from A.AL donors (expressing the same I region disparity), the test grafts were rejected in first-set fashion, indicating that these animals had not been sensitised by their prior experience with the corneal graft (MST of 14.8 and 15 compared with 9.0 d, Table 1, lines 5–6). We conclude that corneas in which the epithelium is essentially devoid of a resident Langerhans cell population are unable to display class II alloantigens in a manner capable of inciting transplantation immunity. We recognise that, as the corneal allografts failed to sensitise to Ia alloantigens, the stromal elements and endothelium of these grafts must also be unable to express these antigens effectively.

Our findings have immediate relevance to clinical corneal transplantation. Even without tissue typing to assist donor selection, corneal allografts have been surprisingly, although not regularly, successful in man¹⁷. Our results suggest that deficiency of Langerhans cells in the cornea may be important in this. HLA-D/DR alloantigens are potent transplantation immunogens in man. If, by extrapolation from the mouse data, human corneas fail to express D/DR determinants, then the alloantigenic burden of cornea is significantly less. Perhaps for the success of corneal transplants in man, matching for HLA-A, B, C may be sufficient. A recent report indicates that donor-recipient matching for HLA-A, B, C corresponds to improved corneal allograft survival¹⁸.

The absence of Langerhans cells from the cornea has other interesting implications. It has been demonstrated that, shortly after vaccinia virus vaccination in man, virtually all viral particles become associated with epidermal Langerhans cells¹⁹. These cells have been ascribed the general property of antigen processing; perhaps the processing and/or handling of cutaneous viral infections is also a function of the dendritic Langerhans cell. Indolent and persistent infections of corneal epithelium often caused by herpes simplex virus may be another expression of the virally related function of this interesting epidermal cell type. The absence of Langerhans cells from the cornea (assuming that they are important in defence against viral infection) would render this epidermal tissue especially vulnerable to persistent and chronic virus infection.

This work was supported in part by USPHS grants AI-10678, EY-03119 and CA09082. We thank Ms Sabra Sullivan for technical assistance. G.B.T. was a postdoctoral fellow sponsored by the Parker B. Francis Foundation.

Received 7 May; accepted 7 September 1979.

1. Klein, J. *Biology of the Mouse Histocompatibility-2 Complex* (Springer, New York, 1975).
2. Hammerling, G. J. *Transplant Rev.* **30**, 64–82 (1976).
3. Hammerling, G., Mauve, G., Goldberg, E. & McDevitt, H. O. *Immunogenetics* **11**, 428–437 (1975).
4. Frelinger, J. G., Wettstein, P. J., Frelinger, J. A. & Hood, L. *Immunogenetics* **6**, 125–135 (1978).
5. Rowden, G., Lewis, M. G. & Sullivan, A. K. *Nature* **268**, 247–248 (1977).
6. Klareskog, L., Tjernlund, U., Forsum, U. & Peterson, P. A. *Nature* **268**, 248–250 (1977).
7. Stingl, G., Katz, S., Shevach, A. M., Wolff-Schreiner, E. & Green, I. J. *Immun.* **120**, 570–578 (1978).
8. Rowden, G., Phillips, T. M. & Delovitch, T. L. *Immunogenetics* **7**, 465–478 (1979).
9. Silberberg, I., Baer, R. L. & Rosenthal, S. A. *J. invest. Derm.* **66**, 210–217 (1976).
10. Shelley, W. B. & Juhlin, L. *Archs Derm.* **113**, 187–192 (1977).
11. Potten, C. S. & Allen, T. D. *Differentiation* **5**, 43–47 (1976).
12. Bergstresser, P., Fletcher, C. & Streilein, J. W. *J. invest. Derm.* (in the press).
13. Bergsloeb, E. *Anals Oculist* **159**, 523–537 (1922).
14. Billingham, R. E. & Medawar, P. B. *Trans. R. Soc. B* **237**, 151–171 (1953).
15. Pei, Y. F. & Rhodin, J. A. G. *Invest. Ophthal.* **10**, 811–825 (1971).
16. Klareskog, L., Forsum, U., Tjernlund, U., Rask, L. & Peterson, P. A. *Invest. Ophthal. visual Sci.* **18**, 310–313 (1979).
17. Silverstein, A. M. & Khodadoust, A. A. *Ciba Fdn Symp.* **15**, 105 (1973).
18. Batchelor, J. R. *et al. Lancet* **i**, 551–554 (1976).
19. Nagao, S., Inaba, S. & Iijima, S. *Archs derm. Res.* **256**, 23–31 (1976).

A defect in the antigen-presenting function of macrophages from neonatal mice

Christopher Y. Lu, Edward G. Calamai & Emil R. Unanue

Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

The newborn mammal has a limited ability to mount an immune response^{1–5}. As the individual matures, the potential for immunological reactivity develops and expands. During the early period of relative immune incompetence, tolerance to some foreign antigens is easily induced^{6,7}. It is thought that the individual may be acquiring tolerance to self antigens during this period, hence its importance for immunological homeostasis. The contribution of mononuclear phagocytes to the initiation of immune responses is now well established. These phagocytes are essential for the development of various lymphocyte functions, in particular, those of the T lymphocytes. Most T-lymphocyte activities require that the macrophage take up and 'present' the antigen in a process modulated by the I region of the major histocompatibility gene complex (for review see ref. 8). Although some earlier studies^{9–13} have indicated deficient macrophage function in the neonate, precise definition of the putative deficiency is lacking. We show here that macrophages from neonatal mice present antigen poorly and that this can be correlated with the small number of macrophages that bear Ia antigens.

In our tissue culture assay system, T lymphocytes from mice immunised to *Listeria monocytogenes* were cultured with macrophages which had taken up heat-killed *Listeria* organisms. The macrophages were always obtained from non-immune mice. It had been previously established that effective interaction between T lymphocyte, macrophage and *Listeria* resulted in T-lymphocyte proliferation¹⁴. Proliferation of the *Listeria*-immune T lymphocytes was antigen specific and required uptake of *Listeria* by a subpopulation of macrophages bearing Ia antigen and homology between T lymphocyte and macrophage at the I-A region of the major histocompatibility gene complex. This assay system is similar to other systems^{8,15} using soluble protein antigens and measures an antigen-presentation function of the macrophage regulated by the major histocompatibility gene complex.

The data in Table 1 demonstrate that culturing *Listeria*-immune T lymphocytes from adult (2–3-month-old) mice with *Listeria*-pulsed macrophages also from adults resulted in T-lymphocyte proliferation. A weak proliferative response was observed when the same number of macrophages from neonatal mice was substituted for the macrophages from adults. Direct microscopic counts established that equal numbers of adherent

Table 1 Comparison of adult immune T-lymphocyte stimulation by *Listeria*-pulsed macrophages from C57BL/6J mice of different ages

<i>Listeria</i> -immune T cells	Age of macrophage donor	Pulse of <i>Listeria</i>	c.p.m.
+	No macrophages	0	1,024 ± 210
+	8–12 weeks	0	577 ± 28
+		+	43,367 ± 1,201
+	4 days	0	647 ± 123
+		+	1,947 ± 170
+	10 days	0	492 ± 20
+		+	3,699 ± 981
+	15 days	0	670 ± 109
+		+	4,803 ± 1,451

Macrophages were obtained from the peptone-elicited peritoneal exudate of non-infected C57BL/6J mice of various ages. Sterile proteose peptone, 10% (75 µl per g body weight) was injected intraperitoneally (i.p.), and the resulting exudate collected 4 d later by lavage with Hank's balanced salt solution containing 2% fetal calf serum (FCS), 10 mM HEPES and 10 U l⁻¹ heparin. Peritoneal exudate cells (10⁴) were incubated on flat-bottom 1.0 × 0.7-cm² Linbro wells for 1 h, and then nonadherent cells were removed by washing the wells three times. The adherent cells were pulsed with heat-killed *Listeria* for 1 h. Unbound *Listeria* was removed by washing the wells five times, after which 4 × 10⁵ *Listeria*-immune T lymphocytes were added to each well in 200 µl of RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine and 5 × 10⁻⁵ M 2-mercaptoethanol. The immune non-adherent T lymphocytes were cultured with the *Listeria*-pulsed macrophages for 4 d at 37°C in humidified 5% CO₂. Each well then received 0.2 µCi ³H-thymidine, and trichloroacetic acid-precipitable counts were measured 18 h later. Results indicate the mean of three cultures with the s.e.m. Further technical details are given in ref. 14. The *Listeria*-immune T lymphocytes were isolated from adult C57BL/6 mice injected i.p. with 10⁴–10⁵ live *Listeria*. Seven days after infection, the mice received 1.5 ml of sterile 10% proteose peptone i.p., and the resulting exudate was collected 3 d later. The T lymphocytes were isolated by removing macrophages through adherence on plastic dishes and nylon-wool filtration. Other experiments from this laboratory have firmly established that T lymphocytes cultured without macrophages but with *Listeria* continuously show no proliferation or a modest proliferative response never exceeding about one-quarter of that shown with macrophage-bound *Listeria*¹⁴. By doing the experiment, as above, pulsing the macrophages with *Listeria* before addition of T cells, we minimise any possible effect of residual macrophages in the T-cell population.

cells from neonatal and adult mice were being compared. The adherent cell population from neonates and adults had the same composition—greater than 99% macrophages as determined by morphology and positive, nonspecific esterase staining. Furthermore, the macrophages from adult and neonatal mice had exactly the same ability to take up *Listeria* and similar distributions of Fc and C3 receptors.

Figure 1 shows that only optimal numbers of macrophages from adults, 10⁴–3 × 10⁴ per microwell, stimulated immune T-cell proliferation. Macrophages from neonatal mice weakly stimulated T-lymphocyte proliferation regardless of the number of macrophages in the microwells. Furthermore, increasing the amount of *Listeria* given to the macrophages from neonatal

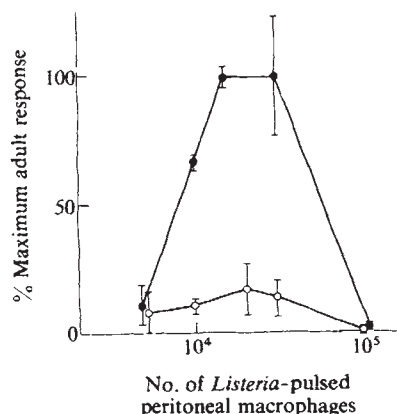


Fig. 1 Immune T-lymphocyte stimulation by antigen-pulsed macrophages as a function of the number of macrophages. The assay of *Listeria*-immune T-lymphocyte stimulation by *Listeria*-pulsed macrophages is described in Table 1 legend. Macrophages from neonatal (○) or adult (●) mice were pulsed with 10⁷ *Listeria* for 1 h. The figure summarises three experiments.

animals did not overcome their deficient ability to stimulate immune T-lymphocyte proliferation (data not shown).

Figure 2 shows that the ability of macrophages to interact effectively with T lymphocytes did not occur until 3–4 weeks after birth, approximately the time of weaning. In each of nine experiments, peritoneal macrophages were taken from C57BL/6J mice of various ages, given *Listeria*, and used to stimulate immune syngeneic T-lymphocyte proliferation. The deficiency detailed above also applied to neonatal splenic adherent cells (not shown). The deficiency was also found in macrophages taken from neonatal mice of a different strain, the A/St strain (H-2^s haplotype).

We have also investigated effects of T-lymphocyte-macrophage interaction other than proliferation. Macrophages secrete a 15,000-molecular weight protein mitogenic for thymocytes^{14,16}. This secretion is optimal when the macrophage, antigen and T lymphocyte interaction occurs with I-A homology between the two cells. Although macrophages from adults secreted the mitogen when mixed with *Listeria*-immune T lymphocytes and *Listeria*, this was not the case with macrophages from neonatal animals (not shown).

There are several possible explanations for the deficient ability of macrophages from neonatal mice to stimulate T-lymphocyte proliferation. First, a possible contamination of the macrophage population with suppressor T cells seems unlikely because of the purity of the macrophage populations. Furthermore, treatment of macrophages with anti-Thy-1.2 and complement did not change the results. Second, a possible nonspecific suppressive influence of macrophages from neonatal mice on lymphocyte proliferation¹⁷ was investigated by mixing macrophages from neonatal animals with syngeneic splenic T lymphocytes stimulated with concanavalin A. Table 2 shows that the macrophages from the neonatal mouse did not suppress the concanavalin A response. In several experiments, we have found that macrophages from adults can stimulate *Listeria*-immune T-lymphocyte proliferation in cultures containing equal numbers of macrophages from neonatal animals. In one example, the response of immune T lymphocytes to 10⁴ macrophages from adults, 10⁴ from neonatal animals and a mixture of 10⁴ cells of both was 19,218, 3,227 and 16,950 c.p.m., respectively.

The inability of macrophages from neonatal mice to stimulate T-lymphocyte proliferation effectively may be due to the small number of Ia-bearing macrophages in the neonatal animals (Table 3). The macrophage population from neonatal mice was deficient in a subpopulation carrying surface Ia antigen. This observation was made with various antisera-target cell combinations. The appearance of Ia-positive macrophages in ontogeny varied from experiment to experiment, perhaps reflecting uncontrollable environmental conditions. It has been

Table 2 Macrophages from neonatal and adult mice do not inhibit concanavalin A-stimulated splenocyte proliferation

Nylon-wool-filtered splenocytes	Age of macrophage donor	Concanavalin A	c.p.m.	
			Expt 1	Expt 2
+	—	0	2,762 ± 87	590 ± 27
+	—	+	53,469 ± 4,736	25,584 ± 996
0	9–11 d	+	348 ± 49	—
+	9–11 d	0	4,540 ± 591	—
+	9–11 d	+	61,732 ± 1,922	30,923 ± 1,821
0	10–12 weeks	+	272 ± 35	—
+	10–12 weeks	0	7,000 ± 860	—
+	10–12 weeks	+	68,270 ± 2,367	41,682 ± 1,469

Macrophages (10⁴ per well) were collected from neonatal or adult mice and pulsed with 10⁷ heat-killed *Listeria* as described in Table 1 legend. Splenocytes were collected from 2–3-month-old C57BL/6J mice and enriched for T lymphocytes by passage over nylon-wool columns. Nylon-wool-filtered cells (4 × 10⁵) were placed in 1.0 × 0.7 cm² Linbro wells containing the *Listeria*-pulsed macrophages. Concanavalin A (4 µg ml⁻¹) was added to the wells. After 4 d of culture, each well received 0.2 µCi ³H-thymidine, and trichloroacetic acid-precipitable counts were measured 18 h later. Similar results were obtained when 5 × 10⁴ instead of 10⁴ macrophages per microwell were used.

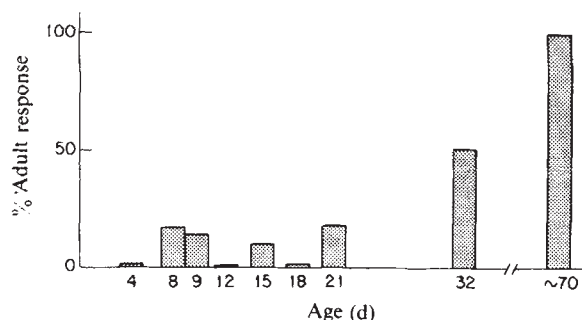


Fig. 2 Ontogeny of immune T-cell stimulation by *Listeria*-pulsed macrophages. Table 1 legend described the assay system for measuring immune T-lymphocyte stimulation by *Listeria*-pulsed macrophages taken from C57BL/6J mice of various ages. The immune T-cell stimulation by macrophages from mice of various ages is expressed as a percentage of the immune T-cells stimulation by macrophages from adult mice. The results were obtained from nine separate experiments.

established in the adult that only Ia-bearing macrophages present *Listeria* and that the cooperation between macrophage and T lymphocyte which allows antigen-driven T-lymphocyte proliferation requires homology between macrophage and T lymphocyte at the I-A region of the major histocompatibility gene complex^{14,16}. Extrapolating from these data, one would predict that a macrophage population with a few Ia-positive cells would present antigen poorly, and this may be the case for the neonatal animal. Whether other factors in the neonatal macrophage population are involved is not known.

Table 3 Detection of Ia antigens on peptone-elicited macrophages

Expt	Strain of macrophage	Age of macrophage donor	Cytotoxic index (% dead)
1	C57BL/6J	8-12 weeks	29
		7 d	9
		10 d	3
2	C57BL/6J	8-12 weeks	25
		8 d	2
		25 d	2
3	C57BL/6J	8-12 weeks	40
		8 d	2
		23 d	29
		38 d	16
4	A.TL	8-12 weeks	29
		10 d	8
		23 d	9
5	C57BL/6	8-12 weeks	28
		12 d	0

Macrophages were isolated from peptone peritoneal exudates by adherence and then incubated for 1 h in antisera at 0°C in flat-bottom 10-mm culture plates. After washing the macrophages at 0°C, these cells were incubated in a mixture of 6% rabbit and 4% guinea pig complement for 1 h at 37°C. The sera had been absorbed extensively with mouse spleen cells to remove natural anti-membrane antibodies. The complement was washed away, and the macrophages were incubated in 0.4% trypan blue in normal saline at room temperature for 15 min. Dead and living cells were scored under a Nikon inverted microscope. Neither dead (trypan-blue-positive) nor living (trypan-blue-negative) macrophages were lost from the microwells during the antiserum-complement treatment. The number of dead macrophages following treatment with normal mouse serum (NMS) plus complement was never greater than 5%. The results are expressed as the cytotoxic index: $[(\% \text{ Ia positive} - \% \text{ NMS positive}) \times 100] / (100 - \% \text{ NMS positive})$. The A.TH-anti-A.TL antiserum used in expts 1 and 2 has been previously used¹⁴ and shown to be Ia specific by appropriate absorptions. Used against C57BL/6J macrophages, this antiserum will detect Ia specificity 3 (ref. 25). The A.TH-anti-A.TL antiserum used in expts 3 and 5 (Cedarlane Laboratories) had also been previously standardised. The antiserum used in expt 4 was raised by injecting (LP $\times$ A.TH)F₁ mice with (LP $\times$ A.TL)F₁ and C157 splenocytes and was absorbed with B10.7R spleen cells. The antiserum should detect Ia specificities 1, 2 and 7 (refs 25, 26). This antiserum did not kill control C57BL/6J spleen cells, which do not have Ia specificities 1, 2 or 7. None of these antisera killed control nylon-wool-purified A/St or C57BL/6J splenic T lymphocytes which lack significant amounts of Ia. All antisera were used at an optimal dilution. The cytotoxic index of macrophages taken from mice of similar ages varies from experiment to experiment. This may be attributed to variation in the rate of development of individual litters.

Our study demonstrates that macrophages from neonatal mice do not present antigen efficiently and that this can be correlated with the small number of macrophages bearing Ia antigens. These results allow us to speculate about the two major characteristics of the neonatal immune response. First, with few antigen-presenting macrophages, the T-lymphocyte responses regulated by the major histocompatibility complex (helper, cellular immunity) would not be induced. This would contribute to the limited immune response capacity of the neonatal mouse. Second, interaction of antigen directly with T lymphocytes in the absence of Ia-positive macrophages may induce antigen-specific suppressor T lymphocytes^{18,19}. This would suggest another mechanism for control of self-recognition and the ease of tolerance induction seen in the newborn. Of course, the macrophage is not the only immature cell in the neonatal animal. B lymphocytes²⁰⁻²³ and T lymphocytes^{5,24}, each with unique properties during early life, have been described and also contribute to the special characteristics of the neonatal immune response.

We thank Kevin Cunningham, Thomas Manning, Joseph Scrima and Willie Washington for their care of the breeding colony. Antiserum used in expts 1 and 2 of Table 3 was from Dr David Sachs, and that used in expt 4 was from Dr Martin E. Dorf. Our work was supported by grants from the NIH and from the Council for Tobacco Research.

Received 17 May; accepted 25 September 1979.

1. Sterzl, D. & Silverstein, A. M. *Adv. Immun.* **6**, 337 (1967).
2. Good, R. A. & Papermaster, B. W. *Adv. Immun.* **4**, 1 (1964).
3. Klinman, N. R., Metcalf, E. S. & Sigal, N. H. in *Development of Host Defenses* (eds Cooper, M. D. & Dayton, D. H.) 93 (Raven, New York, 1977).
4. Sherwin, W. K. & Rowlands, L. T. *J. Immun.* **111**, 1549 (1975).
5. Blaese, R. M. & Lawrence, E. C. in *Development of Host Defenses* (eds Cooper, M. D. & Dayton, D. H.) 201 (Raven, New York, 1977).
6. Smith, R. T. *Adv. Immun.* **1**, 67 (1961).
7. Billingham, R. E. & Brent, L. *Phil. Trans. R. Soc. B* **242**, 439 (1959).
8. Möller, G. (ed.). *Immun. Rev.* **40** (1978).
9. Landahl, C. A. *Eur. J. Immun.* **6**, 130 (1976).
10. Hirsch, M. S., Zisman, B. & Allison, A. C. *J. Immun.* **101**, 1160 (1970).
11. Hardy, B., Globerson, A. & Danon, D. *Cell. Immun.* **9**, 282 (1973).
12. Argyris, B. F. *J. exp. Med.* **128**, 459 (1968).
13. Rager-Zisman, B. & Allison, A. C. *J. gen. Virol.* **19**, 329 (1973).
14. Farr, A. G., Kiely, J.-M. & Unanue, E. R. *J. Immun.* **122**, 2395 (1979).
15. Schwartz, R. H., Yano, A. & Paul, W. E. *Immun. Rev.* **40**, 153 (1978).
16. Farr, A. G., Dorf, M. E. & Unanue, E. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3542 (1977).
17. Allison, A. C. *Transplant. Rev.* **40**, 3 (1978).
18. Ishizaka, K. & Ada, T. *J. Immun.* **117**, 40 (1976).
19. Pierres, M. & Germain, R. N. *J. Immun.* **121**, 1306 (1978).
20. Vitetta, E. S. *et al. J. exp. Med.* **141**, 206 (1975).
21. Raff, M. C. *et al. J. exp. Med.* **142**, 1052 (1975).
22. Sidman, C. L. & Unanue, E. R. *Nature* **257**, 149 (1975).
23. Nossal, G. J. V. & Pike, B. L. *J. exp. Med.* **148**, 1161 (1978).
24. Mosier, D. E. & Johnson, B. M. *J. exp. Med.* **141**, 216 (1975).
25. Shreffler, D. C. & David, C. S. *Adv. Immun.* **20**, 125 (1975).
26. David, C. S. & Shreffler, D. C. *Transplantation* **18**, 313 (1974).

Acetylcholine receptor-controlled ion fluxes in membrane vesicles investigated by fast reaction techniques

George P. Hess, Derek J. Cash & Hitoshi Aoshima

Section of Biochemistry, Molecular and Cell Biology,
270 Clark Hall, Cornell University, Ithaca, New York 14853

The ability of a nerve cell to integrate its response to chemical signals (neurotransmitters) arriving from many other cells is central to the function of the nervous system. This integration process depends on the ability of the neurotransmitters to bind to specific receptors which modulate the flow of specific inorganic ions through the cell membrane, and on the ability of the cell to transmit a (chemical) signal to an adjacent cell¹⁻³ only if the resulting change in transmembrane potential is of appropriate sign and magnitude. The controlling sign and magnitude of the cell transmembrane potential can be predicted from measurement of the rates of movement of specific inorganic ions across the membrane⁴⁻⁶ and their dependence on neurotransmitter concentration. However, there have been two main

obstacles to the development of a suitable preparation for such measurements. First, Kasai and Changeux⁷ obtained a membrane vesicle preparation from the electric organ of *Electrophorus electricus* (electroplax vesicles) in which acetylcholine receptor-controlled fluxes of inorganic ions could be observed, but the flux rates seemed too slow to be of physiological significance⁸. We have shown that these slow flux rates were associated with a large fraction of the vesicle preparation which did not respond to acetylcholine and yet dominated the measurements⁹. We then accomplished the separation of the receptor-controlled fluxes from other processes^{9,10}. Second, the receptor-controlled flux was found to be biphasic, with an initial phase too fast to be measured by available techniques¹¹. We have now succeeded in applying a quench flow technique¹² to the determination of the flux rate of specific inorganic ions across vesicle membranes in the millisecond time region, a time resolution sufficient for measuring the initial phase of receptor-controlled fluxes with these vesicles.

The receptor-controlled fluxes were characterised by obtaining three different kinetic measurements using the quench flow technique. Figure 1a shows rapid receptor-mediated flux of ⁸⁶Rb⁺ into the vesicles in the presence of 1 mM carbamylcholine, a stable analogue of acetylcholine. The influx was stopped after various times by mixing the reaction solution with (+)tubocurarine solution to give a final concentration of 10 mM. The ⁸⁶Rb⁺ content of the vesicles was determined by a Millipore filter assay as previously described⁷. Half of the ⁸⁶Rb⁺ influx occurs in 160 ms and the process is complete within 1 s, no further influx being observed at least within 30 s. The data in Fig. 1b demonstrate that receptor-controlled flux is affected by preincubation of the vesicles with 1 mM carbamylcholine. After the various preincubation times indicated, the vesicles were mixed with ⁸⁶Rb⁺ and influx was allowed to proceed for 6 s in the presence of 1 mM carbamylcholine. In vesicles which had not been preincubated with 1 mM carbamylcholine, influx was complete within this assay period. With vesicles which had first been preincubated with 1 mM carbamylcholine for 450 ms, influx was only 50% complete within the assay period. As Fig. 1c shows, a relatively slow rate of flux is observed after preincubation of the vesicles with 1 mM carbamylcholine for 10 s. After this preincubation, influx of ⁸⁶Rb⁺ in the presence of 1 mM carbamylcholine was measured at the times indicated. The final concentration of ⁸⁶Rb⁺ in the vesicles was the same as in the experiment shown in Fig. 1a, but ~25 s were required for half the ⁸⁶Rb⁺ influx to occur, that is, 150 times longer than when the vesicles had not been preincubated with carbamylcholine.

The model in Fig. 2 was used to evaluate the data shown in Fig. 1. It is based on the observation that acetylcholine receptor-mediated changes in the transmembrane potential of nerve and muscle cells often last less than 1 s, even though acetylcholine or carbamylcholine remains. This decrease in response to neurotransmitter is called desensitisation. To explain the phenomenon, Katz and Thesleff¹³ suggested that the receptor exists predominantly in an active conformation and that this conformation is converted to an inactive form by these compounds. On the basis of the observation that receptor-controlled flux consists of an initial fast phase which is followed by a slow phase¹¹, we suggested that the active conformation is associated with a large flux rate constant (J_1 , Fig. 2), and the inactive conformation with a much smaller one (J_2 , Fig. 2).

The terms of the integrated rate equation pertaining to the model (Fig. 2) are readily evaluated from kinetic measurements because the two phases of the flux occur in separate time regions (Fig. 1). (1) At high concentrations of carbamylcholine the flux associated with the active form of the receptor, indicated by Δ in Fig. 2, dominates the kinetics. The solid line in Fig. 1a was computed using equation (1) (Fig. 2), a β value of 1.5 s^{-1} obtained from the data in Fig. 1b, and a value for $J_1(L_0/L_0 + K_1)$ of 4.8 s^{-1} , which was obtained by a curve-fitting procedure. (2) The disappearance of the fast flux observed in Fig. 1b is a measure of the disappearance of the active form of the receptor. β contains the rate constants, k_{12} , k_{23} , k_{34} and k_{43} , for the

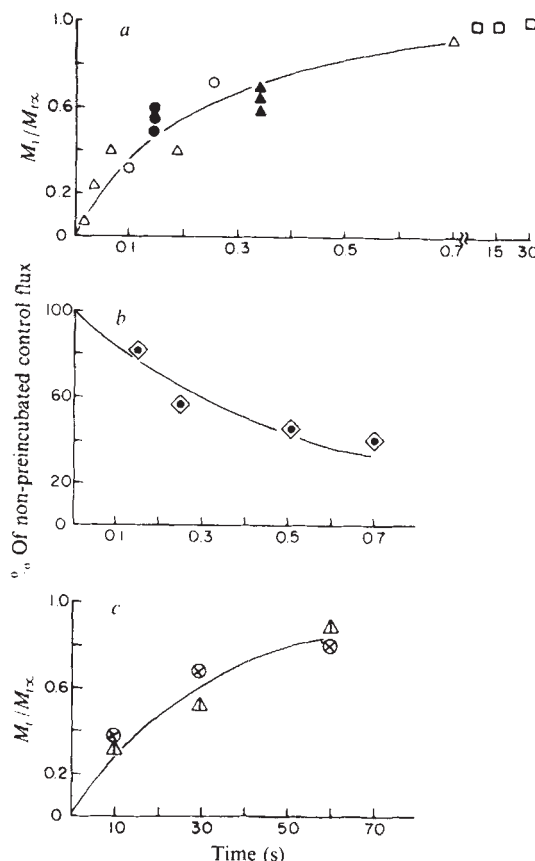


Fig. 1 Acetylcholine receptor-controlled ⁸⁶Rb⁺ flux in electroplax membrane vesicles, pH 7.0, 1°C. *a*, Flux of ⁸⁶Rb⁺ into vesicles in the presence of 1 mM carbamylcholine. [M^*] is proportional to the concentration of ⁸⁶Rb⁺ in the vesicles. The subscript t indicates the time of measurement. *b*, Inactivation of the acetylcholine receptor-controlled ⁸⁶Rb⁺ influx by preincubation of the vesicles with 1 mM carbamylcholine for various periods of time, after which influx was allowed to proceed for 6 s in the presence of ⁸⁶Rb⁺ and 1 mM carbamylcholine. The ordinate gives the results as a percentage of the flux measured when carbamylcholine was absent during preincubation. *c*, Flux of ⁸⁶Rb⁺ into vesicles in the presence of 1 mM carbamylcholine, after preincubation of the vesicles for 10 s with 1 mM carbamylcholine. The different symbols refer to different eel preparations: solid symbols represent single measurements; the symbols in *b* represent the average values obtained in four different experiments using membrane preparations from four different eels. The quench flow apparatus has been described by Fersht and Jakes¹². Vesicles (800 μg membrane protein per ml) were equilibrated for at least 12 h with *E. electricus* Ringer solution¹⁸ (169 mM NaCl, 5 mM KCl, 3 mM CaCl₂, 1.5 mM MgCl₂, 1.5 mM phosphate buffer, pH 7.0). In the influx experiments the vesicles were mixed with an equal volume of eel Ringer solution containing either ⁸⁶Rb⁺ (100 $\mu\text{Ci ml}^{-1}$) or ⁸⁶Rb⁺ and 2 mM carbamylcholine. After the predetermined time 2 volumes of the above solution were mixed with 1 volume of 30 mM (+)tubocurarine chloride which quenched the reaction. The ⁸⁶Rb⁺ content of the vesicles was measured using a Millipore filter assay⁷. The effectiveness of quenching was demonstrated by mixing a solution containing vesicles, carbamylcholine and (+)tubocurarine with a solution containing ⁸⁶Rb⁺ and observing no influx in 5 s, and also by sequentially mixing the vesicles with carbamylcholine and ⁸⁶Rb⁺ and then with (+)tubocurarine and observing no influx. In the desensitisation experiments the vesicles were mixed with an equal volume of 2 mM carbamylcholine or eel Ringer solution. After the predetermined preincubation period 2 volumes of the above solution were mixed with 1 volume of ⁸⁶Rb⁺ and 1 mM or 3 mM carbamylcholine (the final concentration always being 1 mM), and the mixtures incubated for 5 s before Millipore assay. To ensure that vesicle breakage was not significant, the total ⁸⁶Rb⁺ influx measured in the quench flow apparatus was compared with the total influx measured outside the apparatus. The method for isolating the vesicles, the kinetic techniques which allow measurement of the carbamylcholine-induced fluxes of inorganic ions without interference from other fluxes, and the sources of chemicals used, have been described elsewhere⁹⁻¹¹.

isomerisation of receptor conformations¹⁴, and was evaluated from a linear plot of the data according to equation (2) with the concentration of the active form of the receptor at zero time, $[L\Delta]_{t=0}$, set equal to 100% (Fig. 1b). $[L\Delta]_t$ represents the concentration of the active receptor conformation at time t , and is obtained from the ordinate of the graph in Fig. 1b. The solid

line was computed using a value for β of 1.5 s^{-1} . (3) Preincubation of the vesicles with carbamylcholine for 10 s leads to a relatively slow $^{86}\text{Rb}^+$ influx which follows a first-order rate law. The solid line in Fig. 1c was computed using a value for $J_2(L_0/L_0 + K_2)$ of 0.03 s^{-1} . We cannot determine *a priori* which receptor conformation gives rise to this slow flux. If we assume that it arises only from a small concentration of active receptor in an equilibrium mixture of active and inactive forms, $J_2 = K_{c2}J_1$.

Desensitisation of the acetylcholine receptor has previously been observed in flux experiments with electroplax membrane vesicles¹⁴, and in electrophysiological measurements with intact electroplax cells^{15,16}. The suggestion that these observations are due to the isomerisation of receptor conformations, each associated with very different efficiencies in mediating the equilibration of inorganic ions across the cell membrane, is supported by the data in Fig. 1. Furthermore, the overall rate constant for this process, β , measured in flux experiments with electroplax vesicles, is kinetically competent to account for the time course of the electrophysiologically observed desensitisation process in intact, single electroplax cells. The observation that the desensitised form of the receptor also allows inorganic ions to cross the membrane may account for several electrophysiological observations. The extent to which flux rates controlled by particular receptors perturb the transmembrane voltage depends on the extent to which they perturb the relative rates with which inorganic ions move through the cell membrane in the absence of the particular receptors^{4-6,17}. The change in transmembrane potential due to the relatively slow rates associated with the desensitised form of the receptor, is expected to be particularly sensitive to these other fluxes of inorganic ions. Differences in the desensitisation behaviour of different cells, observed in electrophysiological experiments, may therefore reflect characteristics of the cell unrelated to the receptor.

The prediction of the sign and magnitude of the transmembrane potential of a cell, and therefore of the propagation of a signal in a variety of conditions, requires additional measurements. J_1 and J_2 can be evaluated from measurements of the effect of ligand concentration on the flux kinetics. Intrinsic flux rate constants can be determined from a knowledge of the effect of inorganic ion concentrations on J_1 and J_2 , and of the number of receptor sites per vesicle and the internal volume of

the vesicles. The results presented in Fig. 1 show that the technique which we have developed enable the required kinetic measurements to be made.

This work was supported by grants NS08527 and GM04842 from the NIH and grant PCM75-07377 from the NSF. D.J.C. is supported by a Center grant from the NIH (CA14454), H.A. is on leave of absence from Yamaguchi University, Japan. We thank Professor Alan R. Fersht for his advice concerning the building of the quench flow apparatus, and Ms Laura Tenney for her assistance.

Received 9 April; accepted 20 September 1979.

- Hodgkin, A. L. & Huxley, A. F. *J. Physiol., Lond.* **117**, 500-544 (1952).
- Katz, B. *Nerve, Muscle and Synapse* (McGraw-Hill, New York, 1966).
- Nachmansohn, D. & Neumann, E. *Chemical and Molecular Basis of Nerve Activity* (Academic, New York, 1975).
- Nernst, W. *Z. phys. Chem.* **2**, 613-637 (1888).
- Planck, M. *Wied. An.* **40**, 561 (1890).
- Hodgkin, A. L. & Katz, B. *J. Physiol., Lond.* **108**, 37-77 (1949).
- Kasai, M. & Changeux, J. P. *J. Membrane Biol.* **6**, 1-23, 58-80 (1971).
- Katz, B. & Miledi, R. *J. Physiol., Lond.* **224**, 665-669 (1972).
- Hess, G. P., Andrews, J. P., Struve, G. E. & Coombs, S. E. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4371-4375 (1975).
- Hess, G. P. & Andrews, J. P. *Proc. natn. Acad. Sci. U.S.A.* **74**, 482-486 (1977).
- Hess, G. P., Lipkowitz, S. & Struve, G. E. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1703-1707 (1978).
- Fersht, A. R. & Jakes, B. *Biochemistry* **14**, 3350-3362 (1975).
- Katz, B. & Thesleff, S. *J. Physiol., Lond.* **138**, 63-80 (1957).
- Noble, R. L., Epstein, N. & Hess, G. P. *Fedn Proc.* **38**, 513 (1979).
- Del Castillo, J. & Webb, G. D. *J. Physiol., Lond.* **270**, 271-282 (1977).
- Lester, H. A., Changeux, J.-P. & Sheridan, R. E. *J. gen. Physiol.* **65**, 797-816 (1975).
- Hess, G. P. in *The Neurosciences: The Fourth Study Program* (eds Schmitt, F. O. & Worden, F. G.) 847-858 (MIT, Boston, 1979).

A dominant role of thromboxane formation in secondary aggregation of platelets

Kenneth M. Meyers*, Carrie L. Seachord*
Holm Holmsen†, J. Bryan Smith‡ & David J. Prieur§

* Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University

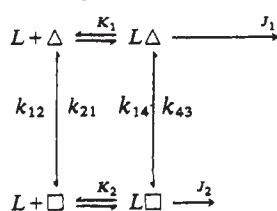
† Specialized Center for Thrombosis Research, Temple University, Philadelphia

‡ Cardeza Foundation and the Department of Pharmacology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

§ Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164

Aggregation of human platelets *in vitro* can occur in two phases—primary and secondary aggregation¹. Primary aggregation is due to the direct interaction of the aggregating agent with its receptor^{2,3}, whereas secondary aggregation is associated with both the secretion of dense granule constituents, including the aggregating agents, serotonin and ADP (ref. 4) and the activation of a pathway for the conversion of arachidonic acid to prostaglandins (PGs)⁵. Both secondary aggregation and the associated secretion of dense granule constituents are contingent on the formation of PGG₂ and PGH₂ which can, in part, be transformed into thromboxane (TX) A₂ (refs 1, 3, 6, 7). TXA₂ is a potent aggregating agent and apparently the functionally active compound in the arachidonate pathway⁸⁻¹⁰. However, the precise role of TXA₂ in mediating secondary aggregation is a matter of controversy as it is not clear whether secondary aggregation can occur independently of secretion. We report here that platelets from cats with the Chediak-Higashi (CH) syndrome¹¹ aggregate with arachidonate in a cyclooxygenase-dependent manner and that biphasic aggregation produced by serotonin is accompanied by TXB₂ formation without detectable ADP secretion. These results demonstrate that secondary platelet aggregation can be mediated through thromboxane formation without participation of secreted ADP.

We have studied the aggregation and secretion of CH cat platelets and simultaneously measured TX formation. Most human patients and all animals, including the cats in this study,



$$(1) \quad \ln \frac{[M^+]_t}{[M^+]_{t=0}} = -\frac{J_1 L_0}{L_0 + K_1} \left(\frac{1 - e^{-\beta t}}{\beta} \right) - \frac{J_2 L_0}{L_0 + K_2} t$$

$$(2) \quad \ln \frac{[L\Delta]_{t=0} - [L\Delta]_t}{[L\Delta]_{t=0}} = -\beta t$$

$$(3) \quad \beta = \frac{k_{12}K_1}{L_0 + K_1} + \frac{k_{34}L_0}{L_0 + K_1} + \frac{k_{21}K_2}{L_0 + K_2} + \frac{k_{43}L_0}{L_0 + K_2}$$

$$(4) \quad K_{c2} = \frac{k_{43}}{k_{34}}, \quad K_{c1} = \frac{k_{21}}{k_{12}}$$

Fig. 2 Minimum mechanism for acetylcholine receptor-controlled ion flux. The conformations referred to as active and inactive by Katz and Thesleff¹³ on the basis of electrophysiological experiments are represented by Δ and $\square$, respectively. J_1 and J_2 are the rate coefficients for ion fluxes associated with the different receptor conformations (Δ and $\square$). Omitted from the scheme for simplicity are the equilibria between the open and closed channel forms of the receptor: ligand complexes, differences in which may be responsible for the differences between J_1 and J_2 . K_1 and K_2 are the ligand dissociation constants pertaining to the two conformations. $[L\Delta]$ in equation (2) represents the concentration of the receptor: ligand complex and the subscript t refers to the time of measurement. β reflects the rate constants for the isomerisation of receptor conformations¹¹. The integrated rate equation published previously¹¹ has been simplified, recognising that the two phases of the reaction occur in widely different time regions.

Table 1 Secretion of ADP and formation of TXB₂ during serotonin (5-HT)-induced aggregation of CH cat platelets

CH Cat no.	Secreted ADP ($\mu\text{mol per } 10^{11}$ platelets)*		TXB ₂ Formed (nmol per 10^{11} platelets)†	
	12.5 μM 5-HT		12.5 μM 5-HT	50 μM 5-HT
			5-HT	5-HT
1	<0.001		0.263	0.554
2	0.004		0.131	0.143
3	0.004		0.087	0.508
Normal	0.248		—	0.465‡

* Total adenine nucleotides (ATP and ADP) and ATP were determined in ethanol-EDTA extracts by a firefly luciferin/luciferase method²³.

† TXA₂ is unstable and rapidly decomposes to TXB₂ (ref. 7) which was measured by radioimmunoassay²⁴.

‡ TXB₂ formation was only assessed in platelets which exhibited serotonin-induced secondary aggregation.

with CH syndrome have an intrinsic platelet defect. In the one human patient studied¹² and all CH animals^{13,14}, there is a virtual absence of serotonin and stored adenine nucleotides. Therefore, aggregation of CH platelets should be independent of secreted ADP and serotonin.

Aggregation responses in platelet-rich-plasma (PRP) from CH cats were monitored with an aggregometer (Chrono-log Corporation). Figure 1 shows that arachidonate induced monophasic irreversible aggregation of CH cat platelets and that this aggregation was completely inhibited by the cyclooxygenase inhibitor indomethacin. Three minutes after the addition of arachidonate the extracellular ADP concentration of normal PRP was 2.14 μM whereas CH PRP contained less than 0.01 μM . These results indicate that metabolites of arachidonic acid can aggregate platelets in the absence of ADP or serotonin secretion and confirms the findings of other workers using rabbit

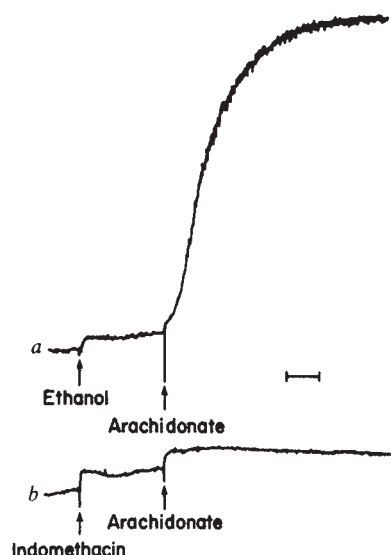


Fig. 1 Aggregation of platelets from CH cat no. 1 to 0.5 mM arachidonate following absolute ethanol treatment ($20 \mu\text{l ml}^{-1}$) (a) and its inhibition by $20 \mu\text{M}$ indomethacin in absolute ethanol ($20 \mu\text{l ml}^{-1}$) (b). Blood samples were withdrawn from the jugular vein from fasted cats anaesthetised with thiamylal sodium. Sodium citrate was used as the anticoagulant (1 part citrate: 9 parts blood). PRP was prepared by centrifugation at 160g at room temperature, and the platelet counts adjusted to 400,000 per μl using autologous platelet-poor plasma (PPP). Scale bar represents 20 s and the aggregation response of a represents 95% of change in light transmission between PRP and PPP. Arachidonate-induced aggregation of feline CH platelets was also inhibited by aspirin ($20 \mu\text{M}$) in sodium acetate, and platelets from normal cats gave monophasic aggregation with 0.5 mM arachidonate (data not shown).

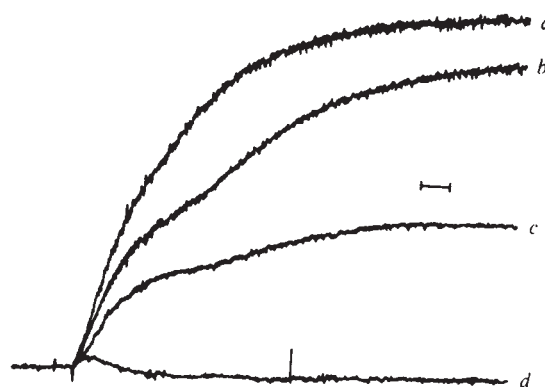


Fig. 2 Aggregation response of platelets from CH cat no. 1 to serotonin. a, 50; b, 12.5; c, 3; d, 1.5 μM . The scale bar represents 20 s and the aggregation response of a is 90% of the change in light transmission between PRP and PPP.

platelets depleted of the constituents of dense granules¹⁵ and human platelets with nucleotide storage pool deficiency¹⁶.

Serotonin is a potent inducer of the aggregation of normal cat platelets¹⁷. The aggregation is biphasic¹⁷ and associated with nucleotide secretion (Table 1). Serotonin also induced biphasic aggregation of CH cat platelets (Fig. 2) which was not accompanied by ADP secretion (Table 1). With increasing concentrations, platelets exhibited first primary reversible aggregation, then biphasic aggregation and finally, monophasic irreversible aggregation. This finding clearly demonstrates that secondary aggregation may occur by processes independent of dense granule secretion.

The serotonin-induced aggregation of CH cat platelets was progressively inhibited by increasing concentrations of three serotonergic receptor antagonists, methysergide, SQ-10,631 and cyproheptadine (Fig. 3). Therefore, both the primary and secondary phases of aggregation seem to depend on the interaction between serotonin and its specific platelet receptor^{2,3}. Although the impulse for the secondary wave of aggregation may have been initiated by the platelet-platelet contact which occurs during serotonin-induced primary aggregation, this seems unlikely as ADP induced primary, but not secondary, aggregation of CH cat platelets (data not shown).

The serotonin-induced secondary aggregation of CH cat platelets required arachidonic acid metabolism. When the formation of PGs was inhibited by treating CH cat platelets with either aspirin or indomethacin, secondary aggregation was abolished (Fig. 4). Furthermore, serotonin caused untreated

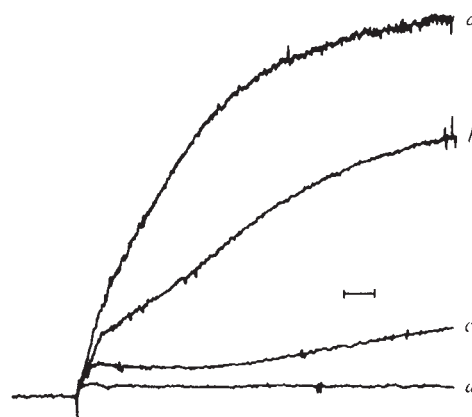


Fig. 3 Aggregation of platelets from CH cat no. 1 to 50 μM serotonin in the presence of increasing cyproheptadine concentrations: a, 0.31; b, 0.63; c, 1.25; d, 10 μM . Scale bar represents 20 s. Aggregation responses of a-d were, respectively, 88, 62, 16 and 1% of the change in light transmission between PRP and PPP. Similar aggregation curves were obtained with both methysergide and SQ-10,631 (data not shown).

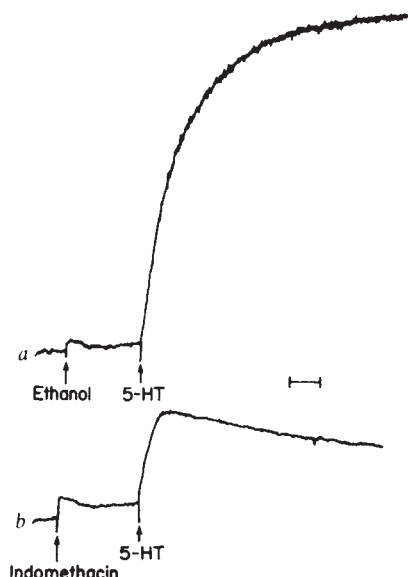


Fig. 4 Aggregation of platelets from CH cat no. 1 to 50 μ M serotonin in the presence of indomethacin (20 μ M) in absolute ethanol. *a*, Represents serotonin-induced aggregation 1 min after absolute ethanol treatment (20 μ l ml⁻¹ PRP). *b*, Represents serotonin-induced aggregation 1 min after indomethacin treatment. Scale bar represents 20 s. Similar aggregation was observed with aspirin (20 μ M) in sodium acetate (data not shown).

normal and CH cat platelets to produce TXB₂ in a concentration-dependent fashion (Table 1).

It has been suggested that secretion precedes secondary aggregation, which is regarded as being due to increased extracellular levels of ADP and serotonin because PG endoperoxides and TXA₂ can induce a rapid release of serotonin and ADP from platelets^{7,18}. This hypothesis is supported by the demonstration of an absence of secondary aggregation of platelets from most, but not all, patients with storage pool deficiency (a group of congenital disorders with a deficiency of platelet dense granules^{19,20}). On the other hand, arachidonic acid, PGG₂ and PGH₂ cause aggregation of platelets from these patients¹⁶ and of normal rabbit platelets depleted of dense granular constituents¹⁵. PGG₂ and PGH₂ can also cause aggregation of normal platelets without demonstrable nucleotide secretion²¹. These results suggest that secondary aggregation may be the result of a direct aggregating effect of TXA₂ and is independent of secretion. Indeed, secondary aggregation frequently precedes secretion and it has been suggested that secretion and secondary aggregation are parallel events which are not causally related²².

It is clear that in the case of serotonin-induced aggregation of platelets from CH cats, secondary aggregation is not the consequence of increased extracellular levels to ADP and serotonin, but is directly related to the formation of TXA₂. The results, therefore, support the hypothesis of Charo and associates²¹ that secondary platelet aggregation can occur independently of secretion. The reason that human platelets from some patients with storage pool deficiency have defective secondary aggregation is not clear. If these platelets are not deficient in cyclooxygenase or thromboxane synthetase, the lack of secondary aggregation could be explained by a positive feedback loop in which secreted substances are required to enhance the formation of TXA₂ to give complete secondary aggregation³.

This study was supported by grants RR00515, HL 24277-01 and K04RR00003 from NIH and 76-742 from the American Heart Association. H.H. is an Established Investigator of the American Heart Association.

2. Mills, D. C. B. & Macfarlane, D. E. in *Platelets in Biology and Pathology* (ed. Gordon, J. L.) 159–193 (North-Holland, New York, 1976).
3. Holmsen, H. *Thromb. Haemostasis* **38**, 1030 (1977).
4. Holmsen, H., Day, H. J. & Setkowsky, C. A. *Biochem. J.* **129**, 67 (1972).
5. Smith, J. B., Ingberman, C., Kocsis, J. J. & Silver, M. J. *J. clin. Invest.* **52**, 965 (1973).
6. Hamberg, M. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3400 (1974).
7. Hamberg, M., Svensson, J. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994 (1975).
8. Gorman, R. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 4007 (1977).
9. Fitzpatrick, F. A., Bundy, G. L., Gorman, R. R. & Honohan, T. *Nature* **275**, 764 (1978).
10. Nicolaou, K. C. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
11. Kramer, J. W., Davis, W. C. & Prieur, D. J. *Lab. Invest.* **36**, 554 (1977).
12. Boxer, G. J. *et al. Br. J. Haemat.* **35**, 521 (1977).
13. Meyers, K. M. *et al. Am. J. Physiol.* (in the press).
14. Meyers, K. M., Holmsen, H., Seachord, C. L., Gorham, J. R. & Prieur, D. J. *Thromb. Haemostasis* **42**, 218 (1979).
15. Kinlough-Rathbone, R. L., Reimers, H. J., Mustard, J. F. & Packham, M. A. *Science* **192**, 1011 (1976).
16. Ingberman, C. M., Smith, J. B., Shapiro, S., Sedar, A. & Silver, M. J. *Blood* **52**, 332 (1978).
17. Tschopp, T. B. *Thromb. Diath. Haemorrh.* **23**, 601 (1970).
18. Malmsten, C., Hamberg, M., Svensson, J. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1446 (1975).
19. Weiss, H. J. & Rogers, J. *Blood* **39**, 187–196 (1972).
20. Holmsen, H. & Weiss, H. J. *Blood* **39**, 197–209 (1972).
21. Charo, I. F. *et al. Nature* **269**, 66 (1977).
22. Charo, I. F., Feinman, R. D. & Detwiler, T. C. *J. clin. Invest.* **60**, 866 (1977).
23. Holmsen, H., Storm, E. & Day, H. J. *Analyt. Biochem.* **46**, 489 (1972).
24. Leory, R. J. *et al. Prostaglandin Med.* **2**, 243 (1979).

Vanadate inhibits (Na⁺ + K⁺)ATPase by blocking a conformational change of the unphosphorylated form

S. J. D. Karlish

Biochemistry Department, Weizmann Institute, Rehovoth, Israel

L. A. Beaugé & I. M. Glynn*

Physiological Laboratory, University of Cambridge, Cambridge, UK

In the presence of extracellular K, intracellular orthovanadate is a potent inhibitor of (Na⁺ + K⁺)ATPase^{1–3}, and because this effect may have a physiological role^{3–6}, it is interesting to ask at which step in the enzyme cycle vanadate acts. In experiments with red cells and squid axons containing high concentrations of ATP, Beaugé and DiPolo^{7,8} found that added vanadate modified the effects of extracellular K, or congeners of K, in a similar way to lowered ATP concentration. They suggested that vanadate might act by retarding the relatively slow, ATP-sensitive, conformational change that is thought to follow the hydrolysis of phosphoenzyme, when that hydrolysis is catalysed by extracellular K (ref. 9). There are several reasons for thinking that the same conformational change (E₂K → E₁K) is the rate-limiting step in the conversion of E₂K to E₁Na that occurs when sufficient Na is added to (Na⁺ + K⁺)ATPase preparations suspended in low-K media in conditions which preclude phosphorylation^{10–14}. The speed of the E₂K → E₁K change, in those circumstances, can be measured by the use of several different fluorescent techniques^{10–12,15}, and we report here experiments with a fluorescein-labelled enzyme to investigate the effects of vanadate. It turns out that, in the same conditions in which vanadate inhibits (Na⁺ + K⁺)ATPase activity, it greatly slows the rate of the conformational change E₂K → E₁K.

Our method takes advantage of the increase in fluorescence that occurs when fluorescein-labelled enzyme changes from the form that exists in Na-free, K-containing media (E₂K), to the form that exists in high-Na media containing little or no K (E₁Na)^{12,15}. The increase in fluorescence is thought to reflect a change in the immediate environment of the fluorescent groups brought about by the change in conformation of the enzyme.

Enzyme prepared from the outer medulla of pig kidney, by the method of Jørgensen¹⁶, was labelled with fluorescein as described in Fig. 1 legend. (The procedure leads to the

* To whom correspondence should be addressed.

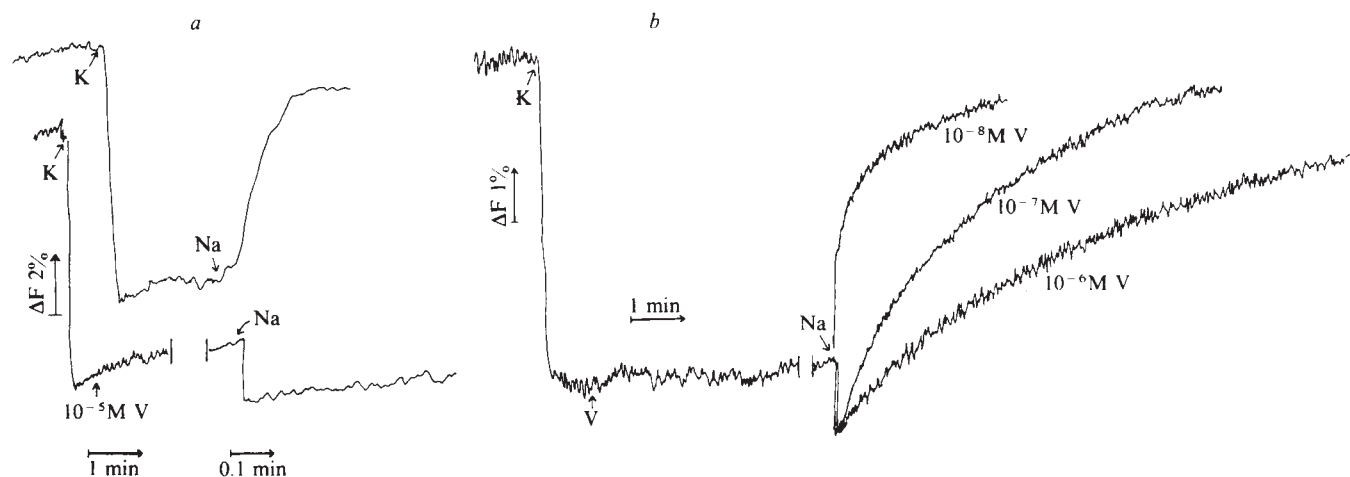
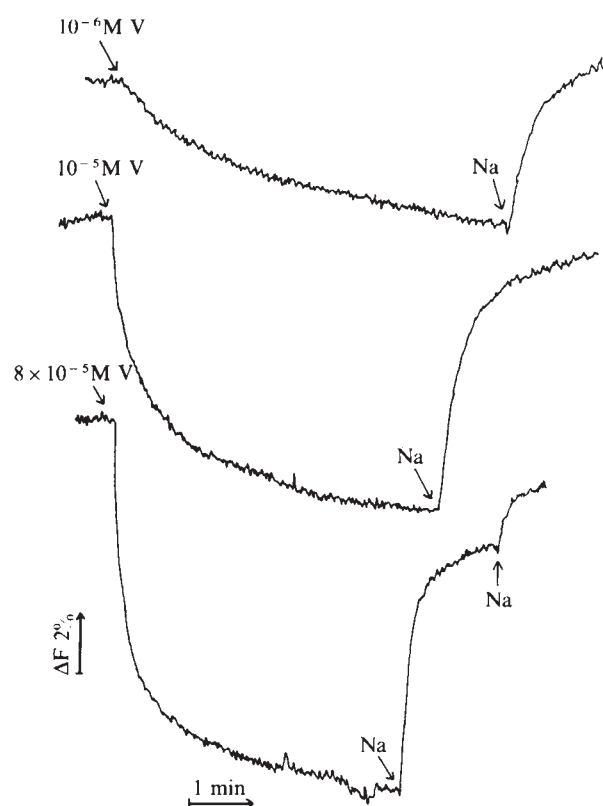


Fig. 1 Ten mg of enzyme (specific activity $20 \mu\text{mol mg}^{-1} \text{min}^{-1}$) was incubated in 3.7 ml of a solution containing 100 mM NaCl, 50 mM Tris/Tris-Cl (pH 9.2 at 20°C) and 5 mM 1,2-diaminocyclohexanetetraacetic acid. Fluorescein was added as a $150 \mu\text{M}$ solution in dried dimethyl formamide to give a final concentration of $7 \mu\text{M}$. The suspension was allowed to stand for 30 min at 24°C and was then dialysed at 0°C against several changes of 150 mM Tris/Tris-Cl (pH 7.5 at 20°C) containing bovine serum albumin (1 mg ml^{-1}) over a period of 48 h. For measurements of fluorescence, $60 \mu\text{g}$ of labelled enzyme was suspended in 2.5 ml of a solution containing 40 mM Tris/Tris-Cl (pH 8.0 at 20°C) and 1 mM MgCl_2 . Excitation was at 495 nm; emission was measured at 520 nm; the slit width was 10 nm, and the time constant of the machine was set at 0.3 s. The temperature was $20 \pm 2^\circ\text{C}$. *a*, Upper trace shows quenching of fluorescence by KCl (final concentration 16 mM) and the reversal of that quenching by NaCl (final concentration 80 mM). The lower trace shows that ammonium vanadate ($10^{-5} M$ final concentration), added after the KCl, blocked the reversal by NaCl. Note (1) the gap in the lower trace represents a period of 5 min, designed to give the vanadate time to act; (2) the time scale was expanded 10-fold just before Na was added; (3) the slit width was 10 nm, and the time constant of the machine was set at 0.3 s. *b*, Traces showing the effects of three different concentrations of vanadate on the rate of rise in fluorescence following the addition of NaCl. For $10^{-6} M$ vanadate, the complete curve is shown; for the other two vanadate concentrations only the part of the curves following the addition of NaCl is shown. Note (1) the gap in the trace represents a period of 5 min, designed to give the vanadate time to act; (2) the time scale was kept constant in these experiments; (3) with $10^{-8} M$ vanadate, the concentration of vanadate was much lower than the concentration of enzyme ($\sim 10^{-7} M$), and the large amount of uninhibited enzyme accounts for the rapid initial rise in fluorescence, and the masking of the expected drop due to dilution.



incorporation of fluorescein groups into the large polypeptide chains of the enzyme, probably near the ATP binding sites. The labelled enzyme is unable to bind or to hydrolyse ATP, but it can still undergo the normal interconversion between E_1 and E_2 forms in response to changes in the concentrations of Na and K^{12,15}.) The fluorescein-labelled enzyme was suspended in a buffered medium, containing Mg but lacking both Na and K, in a fluorimeter cell fitted with a device that allowed additions to be made, and the suspension to be stirred, while the fluorescence was being recorded. In the Na-free, K-free medium, the enzyme was in the E_1 form and the fluorescence was high. When KCl was added to give a final concentration of 16 mM, there was a rapid fall in fluorescence as the enzyme was converted into the E_2K form (Fig. 1). (In the Mg-containing medium the K⁺-induced fall in fluorescence is about 30% smaller than that observed in Mg-free media¹⁵.) Vanadate was then added to give final concentrations in the range 0 – $10^{-5} M$, and was allowed 5 min to act; no significant change in fluorescence occurred during this period. Finally, NaCl was added to give a final concentration of 80 mM. In the absence of vanadate, the fluorescence rose to its original level (allowing for dilution) within 5 s (Fig. 1a). With increasing concentrations of vanadate, the rise in fluorescence was progressively slower (Fig. 1b), until, with $10^{-5} M$ vanadate (Fig. 1a), the addition of Na had very little effect. The inhibition

Fig. 2 The reduction in the fluorescence of fluorescein-labelled enzyme by ammonium vanadate in the absence of KCl, and its reversal by NaCl (80 mM final concentration). The preparation of the enzyme, and the incubation conditions, were identical with those described in the legend to Fig. 1. Note (i) that the range of vanadate concentrations used in these experiments is higher than that in the experiments of Fig. 1; (ii) that ammonium ions were present in much too low a concentration to account for the fluorescence change; (iii) control experiments with Tris/Tris Cl showed that the effect of adding NaCl was not due to the increase in ionic strength.

by vanadate probably reflects the formation of a stable enzyme-K-Mg-vanadate complex⁷.

In a second experiment, ammonium vanadate, in the concentration range 10^{-6} M– 8×10^{-5} M, was added to enzyme suspended in the same Mg-Tris medium in the absence of both Na and K. As shown in Fig. 2, the addition of vanadate, in these conditions, itself caused a drop in fluorescence—faster at higher vanadate concentrations—which could be rapidly reversed by the addition of NaCl. The drop in fluorescence presumably reflected the net conversion of the enzyme to the E_2 form. This must have been caused by the vanadate ions, for, even at the highest concentration of ammonium vanadate, the ammonium ions alone could not have accounted for more than about 5% of the observed change. Furthermore, essentially similar results were obtained when Na vanadate was used. Addition of 80 mM NaCl before the addition of vanadate prevented the fluorescence change.

Inhibition of $(Na^+ + K^+)ATPase$ by vanadate occurs only in the presence of Mg ions¹, which act at the intracellular surface^{5,17}. It is therefore significant that the inhibition of the $E_2 \rightarrow E_1$ conformational change by vanadate could be detected only when Mg ions were present (not shown).

Taken together, the experiments reported here provide strong evidence that vanadate inhibits $(Na^+ + K^+)ATPase$ activity by blocking the $E_2K \rightarrow E_1K$ conformational change that is thought to follow hydrolysis of the phosphoenzyme. When $(Na^+ + K^+)ATPase$ preparations catalyse the hydrolysis of ATP

in the absence of K, the rate limiting step is thought to be the hydrolytic step and not the subsequent conformational change. In the absence of K, therefore, we should expect the hydrolytic activity of the enzyme to be very much less sensitive to vanadate. Such a decrease in sensitivity is, in fact, observed, but the relation between K concentration and the effects of vanadate^{7,8,18} cannot be wholly explained in this way.

We thank Mrs. R. Goldshlegger for technical assistance, and the Medical Research Council for financial support.

Received 3 May; accepted 3 September 1979.

1. Cantley, L. C. *et al.* *J. biol. Chem.* **252**, 7421–7423 (1977).
2. Cantley, L. C., Resh, M. & Guidotti, G. *Nature* **272**, 552–554 (1978).
3. Beaugé, L. A. & Glynn, I. M. *Nature* **272**, 551–552 (1978).
4. Balfour, W. E., Grantham, J. J. & Glynn, I. M. *Nature* **275**, 768 (1978).
5. Cantley, L. C., Cantley, L. G. & Josephson, L. *J. biol. Chem.* **253**, 7361–7368 (1978).
6. Post, R. L. *et al.* in *2nd. Int. Conf. on the Properties and Functions of Na,K-ATPase* (ed. Skou, J. C.) (Academic, London, in the press).
7. Beaugé, L. A. in *2nd. Int. Conf. on the Properties and Functions of Na,K-ATPase* (ed. Skou, J. C.) (Academic, London, in the press).
8. Beaugé, L. A. & DiPolo, R. *Biochim. biophys. Acta* **551**, 220–223 (1979).
9. Post, R. L., Hegyvary, C. & Kume, S. *J. biol. Chem.* **247**, 6530–6540 (1972).
10. Karlsh, S. J. D., Yates, D. W. & Glynn, I. M. *Biochim. biophys. Acta* **525**, 252–264 (1978).
11. Karlsh, S. J. D. & Yates, D. W. *Biochim. biophys. Acta* **527**, 115–130 (1978).
12. Karlsh, S. J. D. *2nd. Int. Conf. on the Properties and Functions of Na,K-ATPase* (ed. Skou, J. C.) (Academic, London, in the press).
13. Glynn, I. M., Karlsh, S. J. D. & Yates, D. W. *2nd. Int. Conf. on the Properties and Functions of Na,K-ATPase* (ed. Skou, J. C.) (Academic, London, in the press).
14. Beaugé, L. A. & Glynn, I. M. *Nature* **280**, 510–512 (1979).
15. Karlsh, S. J. D. (in preparation).
16. Jørgensen, P. L. *Biochim. biophys. Acta* **356**, 36–52 (1974).
17. Beaugé, L. A., Cavieses, J. D., Glynn, I. M. & Grantham, J. J. (in preparation).
18. Beaugé, L. A. & Glynn, I. M. *Nature* **268**, 355–356 (1977).

Purified cardiac cell membranes with high $(Na^+ + K^+)ATPase$ activity contain significant NADH-vanadate reductase activity

Erland Erdmann, Wolfgang Krawietz & Gunther Philipp

Medizinische Klinik I, Klinikum Großhadern, Universität München, D-8000 München 70, FRG

Ingelore Hackbarth, Wilhelm Schmitz & Hasso Scholz

Abteilung III (Biochemische Pharmakologie), Institut für Pharmakologie und Toxikologie, Medizinische Hochschule Hannover, D-3000 Hannover 61, FRG

Frederick L. Crane

Purdue University, Department of Biological Sciences, Lilly Hall of Life Sciences, West Lafayette, Indiana 47907

Vanadium compounds have been reported to inhibit the $(Na^+ + K^+)ATPase$ activity of several cell membrane preparations^{1–3}, to have a positive inotropic effect on ventricular cardiac muscle⁴, slightly increase myocardial cyclic AMP levels⁵, stimulate fat cell adenylate cyclase⁶, and cause natriuresis in rats⁷. Recently we have reported that vanadate produces a transient, initial stimulation of $(Na^+ + K^+)ATPase$ activity in a cat heart cell membrane preparation, in addition to its well known inhibitory action on this enzyme system⁸. The transient stimulation occurred at the same concentration range as did the inotropic effects. There was, however, a serious discrepancy between the time courses of the two events. The former lasted only a few minutes, whereas the latter remained virtually stable for at least 30 min. The supposed stimulatory action of vanadate on $(Na^+ + K^+)ATPase$ was deduced from an initial rapid loss of NADH in the ATPase assay used (optical coupled assay with an ATP regenerating system⁹). The oxidation of NADH was followed photometrically by continuous recording⁸. We now report that the rapid loss of NADH and hence the claimed transient stimulatory action of high concentrations of vanadate on the $(Na^+ + K^+)ATPase$ activity is caused rather by an oxidation of NADH as a result of a NADH-dependent reduction of vanadate.

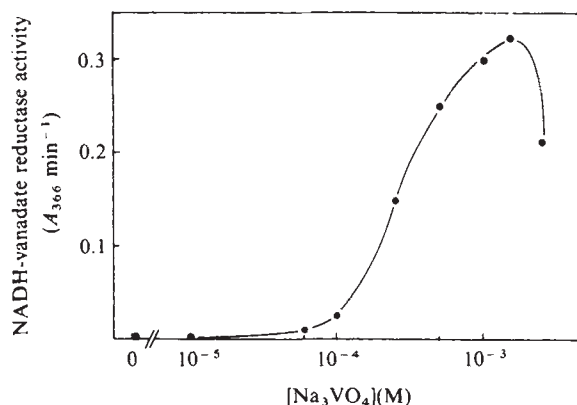


Fig. 1 Effect of vanadate on NADH-reductase activity in a calf ventricular cardiac cell membrane preparation. Calf cardiac cell membranes¹⁰ (0.15 mg protein, 98% of total ATPase activity could be inhibited by 100 μ M ouabain; $(Na^+ + K^+)ATPase$ activity was 1.15 U per mg membrane protein) were incubated at 37 °C in 10 mM imidazole/HCl buffer, pH 7.25, 1.5 mM NADH (Boehringer Mannheim) and the stated concentrations of Na_3VO_4 (freshly prepared). Similar results were obtained with NH_4VO_3 . Total volume 1.6 ml. The oxidation of NADH was followed photometrically at A_{366} by continuous recording. Similar results have been obtained with cell membranes from cat ventricles, human heart and human erythrocytes (not shown). Heating the cardiac cell membranes for 30 min at 60 °C completely destroyed enzyme activity. This curve exhibiting the oxidation of NADH (and reduction of vanadate) has the same concentration dependence as the formerly⁸ reported rapid initial stimulation of NADH oxidation. This demonstrates that the coupled optical assay with NADH often used to measure ATPase activity is seriously influenced by NADH-vanadate reductase activity. The inhibition of enzyme activity at higher vanadate concentrations ($>10^{-3}$ M) might be due to the fact that NADH is oxidised very quickly.

The cardiac cell membrane preparation used in these and the former⁸ experiments was isolated by the method of Pitts and Schwartz¹⁰ but without the glycerol treatment. $(Na^+ + K^+)ATPase$ activity was determined as 0.32 U per mg

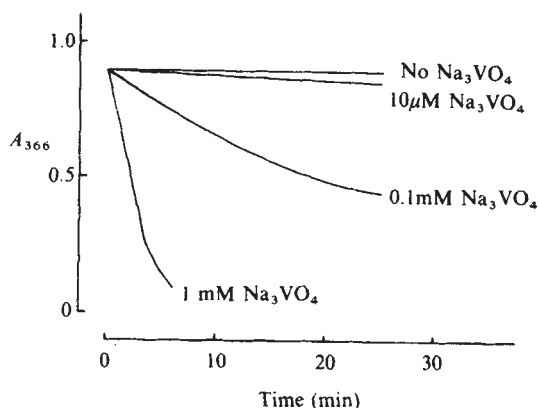


Fig. 2 Time and concentration dependence of NADH oxidation by NADH-vanadate reductase. Calf cardiac cell membranes¹⁰ (0.02 mg protein) were incubated in 10 mM imidazole/HCl buffer, pH 6.75, 1.5 mM NADH and the indicated concentrations of Na_3VO_4 ; total volume 1.6 ml. The experiment was carried out in the same way as in Fig. 1. The curve shows that the oxidation of NADH is linear with time for several minutes.

membrane protein; 97% of total ATPase activity could be inhibited by ouabain (100 μM) and thus was defined as ($\text{Na}^+ + \text{K}^+$)ATPase. One enzyme unit is defined as 1 μmol ATP hydrolysed per min at 37°C. Protein was measured as described by Lowry *et al.*¹¹.

When purified cardiac membranes¹⁰ are incubated with NADH and different concentrations of vanadate but without ATP, a rapid oxidation of NADH, probably due to a reduction of H_2VO_4^- (or $\text{V}_4\text{O}_{12}^{4-}$ or $\text{V}_3\text{O}_9^{3-}$)¹² to VO^{2+} is measured (Fig. 1). Thus, our previous interpretation of a stimulatory action of vanadate on ($\text{Na}^+ + \text{K}^+$)ATPase activity no longer seems justified.

A further analysis shows that the rate of NADH oxidation does depend on the concentration of vanadate (Fig. 2). Furthermore, increasing the amount of membrane protein in the test tubes results in an appropriately increased NADH oxidation (Fig. 3). As there is no NADH oxidation either in the presence of vanadate without membrane protein or in the presence of heat-denatured membranes, the isolated cell membranes must contain a NADH-vanadate reductase. The activity of this enzyme was stable for 4 d when kept at 4°C and lost ~20% activity within 7 d (not shown). The vanadate concentration activating this enzyme half maximally (0.25 mM, Fig. 1) was rather high compared with the concentration of vanadium in serum or tissue (0.1–1 μM)^{1,13}. Thus, unless higher vanadate concentrations at active sites are assumed, the enzyme does not

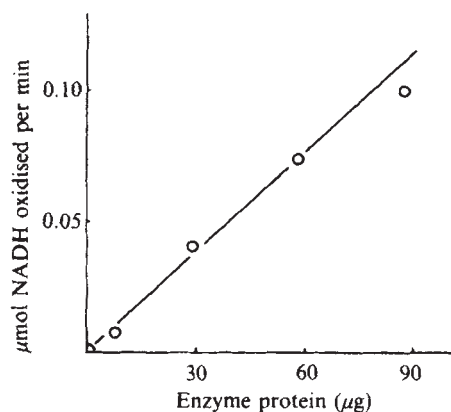


Fig. 3 The oxidation of NADH is increased linearly when the amount of enzyme protein is increased. The experiment was carried out as in Fig. 2 but with increasing amounts of calf cardiac cell membranes¹⁰. There was no NADH oxidation without enzyme.

function at optimal activity in physiological conditions. However, for most enzymes the Michaelis constant (K_m) has been found to be in this range (10–0.01 mM)¹⁴.

As oxidation–reduction mechanisms are widespread in biological systems and have been found in high activities in the plasma membrane, cytoplasm and mitochondrial membrane of a variety of tissues, we think that this NADH-vanadate reductase activity is probably part of the well known plasma membrane NADH-oxidoreductase complex¹⁵. The exact significance of this enzyme for the action of vanadate at the molecular level requires further examination.

It is well known that vanadium compounds exhibit various valence states (II–V) and can be oxidised or reduced easily^{15,16}. It is surprising, however, that purified cardiac cell membranes as used in these experiments contain such high amounts of vanadate reductase activity (about 1.1 U per mg protein). In the light of the results of Cantley *et al.*¹, suggesting that vanadate “may be an ideal specific regulator of ($\text{Na}^+ + \text{K}^+$)ATPase”, this NADH-vanadate reductase in cardiac membranes might have physiological significance. Recently, it has been reported that most of the vanadate in red cells is reduced to vanadyl (VO^{2+}) and bound to haemoglobin¹⁷.

This work was supported by the Deutsche Forschungsgemeinschaft (Er 65/2 and Scho 15/8).

Received 4 June; accepted 31 August 1979.

1. Cantley, L. C. *et al.* *J. biol. Chem.* **252**, 7421–7423 (1977).
2. Beaugé, L. A. & Glynn, I. M. *Nature* **272**, 551–552 (1978).
3. Cantley, L. C., Resh, M. D. & Guidotti, G. *Nature* **272**, 552–554 (1978).
4. Hackbarth, I. *et al.* *Nature* **275**, 67 (1978).
5. Schmitz, W. *et al.* *Naunyn-Schmiedeberg's Archs Pharmacol.* **307**, R37 (1979).
6. Schwabe, U. *et al.* *Nature* **277**, 143–145 (1979).
7. Balfour, W. E., Grantham, J. J. & Glynn, I. M. *Nature* **275**, 768 (1978).
8. Erdmann, E. *et al.* *Nature* **278**, 459–461 (1979).
9. Erdmann, E., Bolte, H.-D. & Lüderitz, B. *Archs Biochim. Biophys.* **145**, 121–125 (1971).
10. Pitts, B. J. R. & Schwartz, A. *Biochim. biophys. Acta* **401**, 184–195 (1975).
11. Lowry, O. H. *et al.* *J. biol. Chem.* **193**, 265–275 (1951).
12. Pope, M. T. & Dale, B. W. *Q. Rev.* **22**, 527–548 (1968).
13. Parker, R. D. R. & Sharma, R. P. *J. envir. Path. Tox.* **2**, 235–245 (1978).
14. Karlson, P. *Kurzes Lehrbuch der Biochemie*, 6th edn, 69 (Thieme, Stuttgart, 1967).
15. Crane, F. L. *et al.* *Subcell. Biochem.* **6**, 345–399 (1979).
16. Rosen, D., Barr, R. & Crane, F. L. *Biochim. biophys. Acta* **408**, 35–46 (1975).
17. Cantley, L. C. & Aisen, P. *J. biol. Chem.* **254**, 1781–1784 (1979).

Alamethicin-induced single channel conductance fluctuations in biological membranes

B. Sakmann* & G. Boheim†

* Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen, FRG

† Ruhr-Universität Bochum, Lehrstuhl für Zellphysiologie, D-4630 Bochum, FRG

Alamethicin, a polypeptide of molecular weight ~2,000, induces voltage-dependent conductance phenomena in artificial lipid bilayer membranes that are in some ways similar to those found in excitable membranes¹. Alamethicin-induced conductance is probably a consequence of the formation of predominantly cation-selective channels which span the bilayer, and single open channels fluctuate at random between several well defined conductance states^{2–5}. Statistical analysis of single channel conductance fluctuations in planar lipid bilayers of various compositions has revealed that, whereas the conductance values are relatively constant in a variety of membranes, the average lifetime of the individual conductance states depends strongly on the lipid used to form the bilayer. This variability has tentatively been attributed to differences in lipid fluidity^{6,7}. Alamethicin is therefore a probe of some aspects of membrane composition. The study of single alamethicin channels in various natural membranes would allow comparison with

the well defined bilayer systems and might yield information on lipid properties of natural membranes that could be important for the functioning of natural membrane channels and receptors. The experiments reported here show that alamethicin channels have similar conductances in the sarcolemmal (surface) membrane of frog and rat muscle and in lipid bilayers formed from synthetic lecithin. The average lifetime of the conductance states is, however, one order of magnitude larger in the biological membranes.

Experiments were carried out on frog (*Rana temporaria*) cutaneous pectoris and rat (Sprague-Dawley) omohyoid muscles bathed in their respective Ringer solutions (for ionic composition see Figs 1 and 3 legends). Alamethicin was applied to a small patch of the extracellular surface of the sarcolemma from a pipette containing alamethicin at nominal concentrations of 9–50 $\mu\text{g ml}^{-1}$. Alamethicin-induced membrane currents were measured by the extracellular patch clamp method^{8,9}. The membrane potential of the muscle fibre was controlled locally by means of a two-microelectrode voltage-clamp circuit.

Figure 1a shows membrane current fluctuations as a function of time, recorded from a patch of frog muscle membrane in the presence of alamethicin at a membrane potential of -110 mV . The tip of the pipette had been in contact with the muscle membrane for $\sim 20\text{ s}$ during which the membrane remained in the resting state. At point A (Fig. 1a), an inward current step is recorded indicating a sudden spontaneous conductance increase of the membrane patch under investigation. The membrane current then fluctuates between six discrete levels of amplitude, some of which occur with a greater probability than others. At point B the current returns to the resting level. This type of

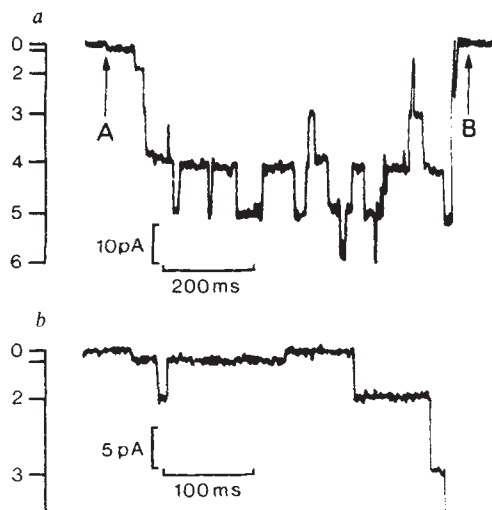


Fig. 1 Alamethicin-induced current fluctuations as a function of time. Frog sarcolemmal membrane at 18°C . Inward current is downwards. The muscle was bathed in frog Ringer solution of the following composition (in mM): Na^+ 118; K^+ 2.5; Ca^{2+} 1.8; Cl^- 121; HPO_4^{2-} 2.15; H_2PO_4^- 0.5; pH 7.2. Alamethicin ($9\text{ }\mu\text{g ml}^{-1}$) dissolved in frog Ringer was applied to the extracellular side of the surface membrane through a patch-pipette with a tip diameter of approximately $2\text{ }\mu\text{m}$. The pipette had a d.c. resistance of $2.5\text{ M}\Omega$. The seal resistances when the pipette was in contact with the muscle were $12\text{--}20\text{ M}\Omega$. This results in a slight underestimation ($\leq 15\%$) of the true current amplitudes⁹. **a**, Oscilloscope traces of a current 'burst' at -110 mV membrane potential. At the time indicated by the arrow labelled A, an inward membrane current turns on, fluctuates for about 1 s between different amplitude levels and at the time indicated by the arrow labelled B, returns to the resting level. Six discrete levels of current amplitude are discernible. The different levels are indicated by the numbered scale on the left side. The level marked 0 corresponds to the resting membrane. **b**, Oscilloscope traces of the initial part of another current burst shown at a faster time base, and higher gain so as to illustrate more clearly the size of the first, second and third current levels. The membrane potential was clamped to -110 mV . The different levels are indicated by the numbered scale on the left side. Resting membrane corresponds to level 0. Both records were low pass filtered at 200 Hz with Bessel characteristics. For analysis of current step sizes and durations, records were stored on magnetic tape. They were replayed on a pen writer at reduced speed. Step sizes and durations were measured by hand and then analysed on a PDP 11/34 computer.

Table 1 Relative distribution probabilities, P_v (%) and mean lifetimes, τ_v (ms) of alamethicin conductance states in natural and synthetic membranes

Conductance state	Frog sarcolemma ($-70\text{ mV}/18^\circ\text{C}$)		Rat sarcolemma ($-65\text{ mV}/20^\circ\text{C}$)		Rat sarcolemma ($-60\text{ mV}/30^\circ\text{C}$)		Dioleylecithin bilayer ($-90\text{ mV}/18^\circ\text{C}$)	
	P_v	τ_v	P_v	τ_v	P_v	τ_v	P_v	τ_v
1	—	—	—	—	39	50	9	1.5
2	10	40	74	130	27	35	20	2.8
3	26	35	19	70	16	35	37	2.4
4	41	40	5	45	12	30	21	2.7
5	19	25	2	40	5	25	6	2.5
6	3	20	—	—	1	10	—	—
7	1	20	—	—	—	—	—	—

In experiments on muscle fibres the pipette contained $9\text{ }\mu\text{g ml}^{-1}$ alamethicin. Numbers in parentheses refer to membrane potential and temperature. The relative distribution probability was calculated by dividing the sum of durations of each current level by the total duration of the current bursts. The mean lifetime was obtained from the decay constant of the distribution of durations at each level fitted by a single exponential. P_v and τ_v values represent means obtained from at least 50 individual events obtained from bursts at the same membrane patch. The beginning of a single burst was defined by the appearance of a spontaneous current jump from the resting level as shown in Fig. 1a. The end of a burst was given either by the return of membrane current to the resting level or by the appearance of a second pore. At membrane potentials of -60 to -70 mV burst durations are longer than at higher membrane potentials and up to 1,500 amplitude transitions occur during a single burst. However, the resting and the first conductance states are not always unambiguously discernible at these potentials. We therefore had to start the statistical analysis with the second conductance level. In experiments with artificial membranes, synthetic dioleylecithin bilayers were used to form the bilayer according to the Montal-Mueller technique¹⁷. The ionic composition of the solutions in the two compartments on both sides of the bilayer was chosen so as to mimic the ionic composition of the extra- and intracellular fluid in frog muscle experiments. The two compartments contained the following ions (in mM): compartment A: Na^+ 119.8; K^+ 2.5; Ca^{2+} 1.8; Cl^- 121.1; HEPES 4.8 (comparable to the extracellular bathing solution in muscle experiments). Compartment B: Na^+ 10.2; K^+ 140; Cl^- 2.2; HEPES 148 (comparable in its main cation composition to the intracellular composition of frog muscle¹⁸). HEPES was chosen to account for the large impermeable intracellular anions of muscle fibres. Compartment B was voltage clamped to -90 mV relative to compartment A. Alamethicin was added to compartment A at $0.5\text{ }\mu\text{g ml}^{-1}$. The reversal potential for alamethicin-induced currents in this system was -38 mV , comparable to the experiments with frog muscle. The sequence of conductance states in this system is (in pS): $\Lambda_1 = 20$; $\Lambda_2 = 110$; $\Lambda_3 = 250$; $\Lambda_4 = 430$; $\Lambda_5 = 620$. (Recently, it has been found¹⁹ that the lowest conductance state of the alamethicin pore in 1 M KCl at 21°C is found at 19 pS . This would correspond to $\sim 1.5\text{ pS}$ in the experimental conditions given in this study and is not resolvable by the present methods.)

current fluctuation closely resembles the pattern seen in lipid bilayer experiments in the presence of low alamethicin concentrations and has been ascribed to the formation of a single pore structure, undergoing transitions between different conductance states and then disappearing^{4,5,10,11}. Current bursts like that shown in Fig. 1a occur at irregular intervals. The times between single bursts vary between a few seconds and about 1 min . It is difficult to investigate interval durations systematically because in most experiments after a few minutes, two or more alamethicin channels become active. This could be due to a continuous increase in alamethicin concentration at the membrane surface. It precludes single channel measurements from the same membrane patch over long periods.

Experiments with artificial bilayers have shown that the discrete current levels occurring during a burst are not integral multiples of a unit current step²⁻⁴. Figure 1b shows clearly that this is also the case in the muscle membrane, as the difference in amplitude between neighbouring current levels increases with the higher levels. Each current level is thought to reflect a discrete conductance state, v , of the alamethicin channel. The different conductances, Λ_v , of the channel in muscle sarcolemma were obtained by measuring current bursts at various membrane potentials and plotting the current-voltage relationship for the different states (Fig. 2). The relationship is approximately linear for all pore states in the range of membrane potentials measured. Extrapolation yields a reversal potential around -40 mV . The pore states vary over nearly two orders of magnitude in their conductance. In frog sarcolemma the Λ_v values are in consecutive order (in pS): $\Lambda_1 = 20$, $\Lambda_2 = 100$, $\Lambda_3 = 260$, $\Lambda_4 = 460$, $\Lambda_5 = 660$, $\Lambda_6 = 860$. For comparison between channels in

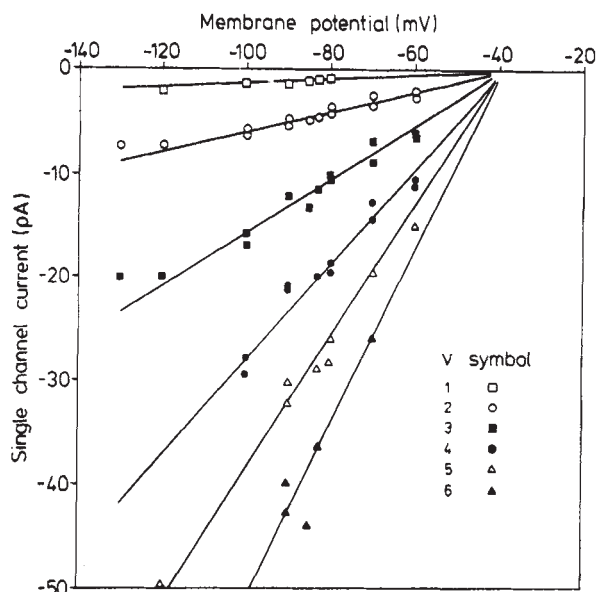


Fig. 2 Current-voltage relationships of the various conductance states, ν , of a single alamethicin channel in frog sarcolemma. The ordinate plots the average size of the inward membrane current during the individual conductance states (indicated by different symbols) as a function of membrane potential. Same experimental conditions as described in Fig. 1 legend with data pooled from two patches. The membrane potential was initially clamped to -130 mV until a current burst occurred. The potential was then shifted down stepwise to -60 mV. At each membrane potential the average size of the different discrete current levels occurring during a burst was determined from the amplitude distribution of the current burst. At each potential except at -60 mV, the level with the smallest average size was attributed to conductance state $\nu = 1$, the next larger level to state $\nu = 2$ and so on. At -60 mV membrane potential the average amplitude of the lowest level could not be measured accurately enough, therefore amplitude analysis was restricted to the higher states. The reversal potential of the alamethicin-mediated current obtained by linear extrapolation is near -40 mV. The conductance values, Λ_ν , of individual states ν obtained from the current-voltage relationships are (in pS): $\Lambda_1 = 20$; $\Lambda_2 = 100$; $\Lambda_3 = 260$; $\Lambda_4 = 460$; $\Lambda_5 = 660$; $\Lambda_6 = 860$. Similar experiments in rat muscle also gave a linear current-voltage relationship extrapolating to a reversal potential near -35 mV. The sequence of conductance values in rat (at 20°C) is (in pS): $\Lambda_1 = 27$; $\Lambda_2 = 125$; $\Lambda_3 = 340$; $\Lambda_4 = 590$; $\Lambda_5 = 840$; $\Lambda_6 = 1,090$.

synthetic and muscle membrane, we have measured, in comparable experimental conditions (see Table 1 legend), alamethicin channel conductances in black lipid films made from synthetic lecithin. The Λ_ν values in this membrane are (in pS): $\Lambda_1 = 20$, $\Lambda_2 = 110$, $\Lambda_3 = 250$, $\Lambda_4 = 430$, $\Lambda_5 = 620$. The conductance sequences of alamethicin channels in the lipid bilayer and in muscle sarcolemma agree quite well. This similarity suggests that bursts like that shown in Fig. 1a are due to conductance fluctuations of a single alamethicin pore, as has previously been established in bilayer experiments²⁻⁴.

The relative stability of the conductance states and the rates of transition between different states were evaluated by measuring relative distribution probabilities, P_ν , of each conductance state and their respective mean lifetimes, τ_ν , according to Boheim⁴. Table 1 shows the P_ν and τ_ν values of alamethicin pore states in frog muscle at 18°C and -70 mV membrane potential. The channel adopts the three most probable states during $\approx 80\%$ of the burst duration, the most stable state being the fourth one with a mean lifetime of 40 ms. For comparison, corresponding values of P_ν and τ_ν are given for the alamethicin channel in the lecithin bilayer. The distribution probabilities of the different pore states are comparable in both frog sarcolemma and lecithin bilayer, although the width of pore state distributions is somewhat narrower in the bilayer. However, there is a large difference in the average lifetimes of the pore states in the two membranes, the means of the τ_ν values of the three most probable states differing by more than one order of magnitude.

The difference in the open-close kinetics of the sodium channel¹² reported for frog and rat muscle prompted us to

compare alamethicin channel state kinetics in both types of muscle. Figure 3a shows a recording of current through a patch of rat sarcolemmal membrane in the presence of a single conducting alamethicin pore. The conductances of corresponding pore states are somewhat larger in rat than in frog muscle, as would be expected from the different Ringer solutions (see Fig. 2 legend). The average lifetimes of the lower pore states in rat are much longer than in frog muscle (Table 1).

As indicated in Fig. 3b, pore state lifetimes are strongly temperature dependent. At 30°C , the lifetimes of all conductance states are shorter than at 20°C and are comparable to the values found in frog muscle at 18°C (Table 1). The activation energies for channel closing were calculated from Arrhenius plots of the means of τ_ν values of the three most probable states. In frog (range 12 – 21°C) the activation energy is ~ 40 kcal mol⁻¹, and in rat (range 15 – 30°C), ~ 20 kcal mol⁻¹. These values are larger by a factor of 4 and 2, respectively, than those reported for artificial membranes^{11,13}.

Thus, the above experiments show that alamethicin induces ion-conducting channels in muscle sarcolemmal membrane which show many properties that are qualitatively similar to those described for alamethicin channels in synthetic lipid bilayers. This suggests that the molecular mechanisms underlying channel formation are similar in both types of membrane. The main difference between the lecithin bilayer and the sarcolemmal membranes lies in the rates of transitions between the different conductance states. The major lipid components of sarcolemmal membrane are lecithins and cholesterol¹⁴, and in lipid bilayer experiments, pore state lifetimes can increase with cholesterol content of the bilayer⁶. Therefore, the long lifetimes in sarcolemmal membrane may be due to the high cholesterol content. The lower rates of conductance state transitions in rat are in agreement with a similar (approximately two- to threefold) difference in sodium channel kinetics between frog and rat muscle fibres^{12,15,16}. This could indicate that some difference between frog and rat sarcolemma, such as membrane viscosity, is responsible for the difference in channel kinetics. By measuring alamethicin channel kinetics in natural membranes in various conditions it might be possible to gain some insight into how membrane lipids can affect the kinetics of naturally occurring ionic channels.

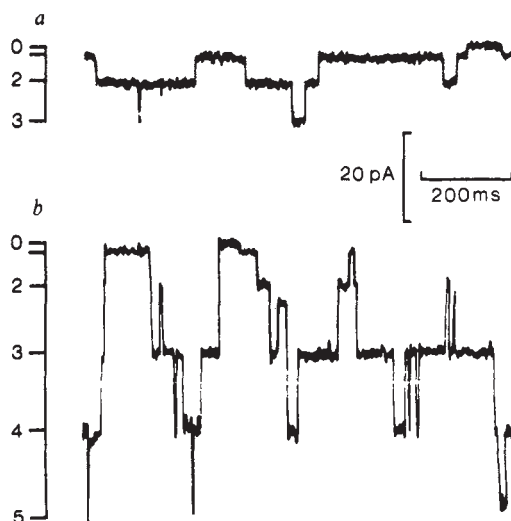


Fig. 3 Current fluctuations induced by alamethicin in rat sarcolemmal membrane at -95 mV membrane potential. Pipette contained $9 \mu\text{g ml}^{-1}$ alamethicin. Inward current is downwards. Muscle is bathed in rat Ringer solution of the following composition (in mM): $\text{Na}^+ 156.4$; $\text{K}^+ 2.8$; $\text{Ca}^{2+} 1.0$; $\text{Mg}^{2+} 2.0$; $\text{Cl}^- 147.4$; $\text{HPO}_4^{2-} 8.2$; $\text{H}_2\text{PO}_4^- 1.4$; pH 7.2. **a**, Oscilloscope record of current fluctuations at 20°C . Numbered scale on the left side indicates discrete current levels. Resting membrane level is indicated by 0. Note longer durations of individual current amplitude levels as compared with those observed in frog muscle at comparable temperature. **b**, Oscilloscope record of fluctuations at 30°C . Calibrations are the same for both **a** and **b**.

This work was financially supported by the Deutsche Forschungsgemeinschaft (S.F.B. 114). Alamethicin was provided by Dr G. B. Whitfield (Upjohn) and dioleylecithin was a gift of K. Janko and Professor P. Luger.

Received 6 August; accepted 12 September 1979.

1. Mueller, P. & Rudin, D. O. *Nature* **217**, 713–719 (1968).
2. Gordon, L. G. M. & Haydon, D. A. *Biochim. biophys. Acta* **255**, 1014–1018 (1972).
3. Eisenberg, M., Hall, J. E. & Mead, C. A. *J. Membrane Biol.* **14**, 143–176 (1973).
4. Boheim, G. *J. Membrane Biol.* **19**, 277–303 (1974).
5. Gordon, L. G. M. & Haydon, D. A. *Phil. Trans. R. Soc. B* **270**, 433–447 (1975).
6. Gordon, L. G. M. & Haydon, D. A. *Biochim. biophys. Acta* **436**, 541–556 (1976).
7. Mueller, P. *Ann. N.Y. Acad. Sci.* **264**, 247–265 (1976).
8. Neher, E. & Sakmann, B. *Nature* **260**, 799–802 (1976).
9. Neher, E., Sakmann, B. & Steinbach, J. H. *Pflugers Arch. ges. Physiol.* **375**, 219–228 (1978).
10. Baumann, G. & Mueller, P. *J. supramolec. Struct.* **2**, 538–557 (1974).
11. Boheim, G. & Kolb, H. A. *J. Membrane Biol.* **38**, 99–150 (1978).
12. Pappone, P. *Biophys. J.* **17**, 3a (1977).
13. Kolb, H. A. & Boheim, G. *J. Membrane Biol.* **38**, 151–191 (1978).
14. Fiehn, W., Peter, J. B., Mead, J. F. & Gan-Elepano, M. *J. biol. Chem.* **246**, 5617–5620 (1971).
15. Duval, A. & Loty, C. *J. Physiol., Lond.* **278**, 403–423 (1978).
16. Adrian, R. H. & Marshall, M. W. *J. Physiol., Lond.* **268**, 223–250 (1977).
17. Montal, M. & Mueller, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3561–3566 (1972).
18. Hodgkin, A. L. & Horowicz, P. *J. Physiol., Lond.* **148**, 127–160 (1959).
19. Hanke, W. & Boheim, G. *Ber. Bunsenges.* (in the press).

Endogenous viral genes are non-essential in the chicken

Susan M. Astrin*†, Edward G. Buss‡
& William S. Hayward§

* Institute for Cancer Research, Fox Chase Cancer Center, 7701
Burholme Avenue, Philadelphia, Pennsylvania 19111

‡ Department of Poultry Science, Pennsylvania State University,
University Park, Pennsylvania 16802

§ The Rockefeller University, New York, New York 10021

DNA sequences homologous to the genomes of type C retroviruses are widespread among vertebrates¹. Ten genetic loci containing endogenous viral DNA sequences have been documented in the white Leghorn chicken alone^{2,3}. Six of these genetic loci are associated with the production of virus or of viral proteins in embryonic fibroblasts (refs 2–4, and S.M.A., L. B. Crittenden and E.G.B., in preparation) and one of the loci may be expressed in the erythroblasts of 5-day-old embryos⁵. The ubiquitous presence of endogenous viral genes among vertebrate species and the association of their expression with development of the haematopoietic system in the mouse⁶ have led to the proposal that these genes are involved in ontogeny. In addition, the genes may be implicated in oncogenesis as in the case of the AKR mouse in which a high incidence of spontaneous leukaemia is associated with the expression of endogenous murine leukaemia virus genomes^{7,8}. We report here the production of a fertile rooster which lacks avian leukosis virus-related endogenous viral genes and which seems to be completely normal and healthy. Thus, endogenous viral genes are apparently not essential for the normal development of the chicken. An endogenous virus-free state has also been reported for three species of jungle fowl⁹ and for the B-type viral genes of the mouse¹⁰.

The DNA of individual chickens was assayed for the presence of endogenous viral sequences by cleavage of embryo or erythrocyte DNA with restriction endonuclease *Sac*I, electrophoretic separation of the resulting fragments, transfer of the fragments, after denaturation, to nitrocellulose filters¹¹, and detection of viral sequences by hybridisation with ³²P-labelled 70S RAV-2 RNA. Endonuclease *Sac*I (an isoschizomer of *Sst*I (ref. 12)) was chosen because it gives a single major band for each site of residence of viral genes in the chicken genome².

Most chickens analysed were homozygous for an endogenous viral locus previously designated as *ev* 1 (ref. 2). However, during a mating experiment involving chickens of defined genotype, we identified two matings which gave rise to a small fraction of embryonic progeny in which no endogenous proviruses could be detected. Twenty-one progeny from these matings were hatched, and DNA was extracted from erythrocytes and assayed for viral sequences. The fragment patterns for the dams, sires and 21 progeny of the two matings are shown in Fig. 1. The bands corresponding to molecular weights of 5.8, 3.7 and 12 × 10⁶ each represent an individual site of residence of endogenous viral genes and have been designated *ev* 1, *ev* 2 and *ev* 5 in accordance with previous nomenclature². As can be seen in Fig. 1, the dams each carried *ev* 1 and *ev* 5 (lane a) whereas the sires carried *ev* 1 and *ev* 2 (lane b). Of the 21 progeny, 16 inherited *ev* 1, 12 inherited *ev* 2, and 11 inherited *ev* 5. One of the progeny (no. 15) apparently inherited no endogenous viral loci. These data are consistent with the sires being heterozygous for both *ev* 1 and *ev* 2 and the dams being heterozygous for both *ev* 1 and *ev* 5. In this case, half the progeny (on average) would be expected to inherit *ev* 2, half would inherit *ev* 5, three-quarters would inherit *ev* 1 and one-sixteenth would be expected to inherit no endogenous viral loci. These figures are very close to the actual frequencies of inheritance observed in the progeny.

To test further the DNA of rooster no. 15 for endogenous viral genes, radiolabelled DNA probes complementary to RAV-0 RNA, the *src* gene, and total cellular DNA were prepared and annealed to the DNAs of chicken 15 and chicken 17. As shown in Fig. 2, the probe to total cellular DNA, the *src* gene probe and the RAV-0 probe extensively to the DNA of chicken 17. The differences in the final percentage of each probe that anneals are most probably due to differences in the sizes of the cDNA probes (see Fig. 2 legend). The DNA of chicken 15 shows normal annealing to the cellular DNA probe and to the *src* gene probe, but shows little or no annealing to the RAV-0 probe. This result is consistent with the DNA of chicken 15 containing less than 2% of the sequences of RAV-0. The presence of *src*-related sequences in the absence of RAV-0 sequences is consistent with the observation that the endogenous *sarc* gene segregates independently of endogenous viral structural genes¹³.

As a more sensitive test for the presence of RAV-0 sequences in the DNA of chicken 15, cDNA probes corresponding to the *gag*, *pol* and *env* genes of ALV and to the 5' 101 nucleotides and the 3' 600 nucleotides ('c' region) of RAV-0 were prepared and annealed to the DNAs of chicken 15 and chicken 17. As shown in Table 1, the DNA of chicken 17 anneals extensively to the five

Table 1 Per cent cDNA hybridised in chickens 15 and 17

Probe*	No. 15	No. 17
cDNA _S	2	66
cDNA _{gag}	0	61
cDNA _{pol}	1	57
cDNA _{env}	1	51
cDNA _c	1	67

Hybridisation was carried out to a C₀t of 3.5 × 10⁴ mol s⁻¹ as described in Fig. 2 legend. All values were corrected for a background of 1–2% with heterologous (salmon sperm) DNA.

* cDNA probes, corresponding to the *gag*, *pol* and *env* genes, were prepared from RAV-2 cDNA. Isolation and characterisation of these probes have been described previously¹⁹. ³²P-labelled cDNA_S (specific activity 4 × 10⁸ c.p.m. per μg) contains sequences complementary to the 101 nucleotides at the 5' terminus of RAV-0 RNA. The DNA was prepared as described by Haseltine *et al.*²⁰. cDNA_S was purified on 10% acrylamide gels, first with the primer tRNA attached, and then after removal of the primer by alkaline hydrolysis. cDNA_c was prepared from RAV-0 cDNA by two cycles of selection against short (9–11S) poly(A)-containing fragments of RAV-0 RNA¹⁹, and thus corresponds to the 500–600 nucleotides adjacent to the poly(A) tract at the 3' end of the RAV-0 genome.

† To whom correspondence should be addressed.

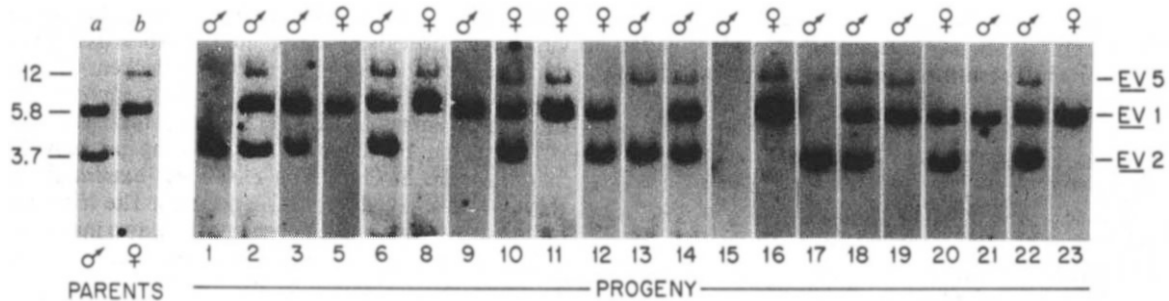


Fig. 1 *Sac*I digestion of chicken DNAs and identification of endogenous viral sequences. A marker of *Eco*RI digested λ 32 P-DNA was used to determine MWs, which are shown $\times 10^{-6}$. Chicken DNAs were prepared² from erythrocytes and digested with *Sac*I. The resulting fragments were separated by electrophoresis on 1% agarose gels² and denatured and transferred to nitrocellulose filters by the method of Southern¹¹. Viral sequences were detected by hybridisation with 70S 32 P-RAV-2 RNA as previously described². The dams used in these matings were *gs⁻chf⁻* hens from SPAFAS line 11. The sires used in the mating were F₁ progeny from a cross between a line 7₂ rooster and *gs⁻chf⁻* hens from SPAFAS line 11.

probes, whereas the DNA of chicken 15 shows little or no annealing to any of the probes. From these results we conclude that chicken 15 definitely lacks avian leukosis virus-related endogenous viral genes.

We are now breeding rooster 15 to his female siblings and to the original dams to obtain stock to be used as the basis of a line of white Leghorns lacking endogenous viral genes. Twenty-one progeny have been hatched and are being tested for endogenous viral loci. One-quarter (on average) of the female siblings of rooster 15 should be homozygous for *ev* 1 because both the dam

and sire of these chickens carried *ev* 1 in the heterozygous state. Matings with these homozygous females will produce no progeny lacking endogenous virus; the remainder of the matings should produce some of this type of progeny. Individuals lacking viral genes should be extremely useful in future studies on the possible involvement of these genes in ontogeny and oncogenesis. In addition, such chickens would provide a host for studying exogenous virus infection in the absence of endogenous proviral information.

The existence of an individual lacking endogenous viral genes implies that these genes are not essential in the chicken. How, then, does one account for their ubiquitous presence? The fact that domestic chickens lacking these genes have not been previously identified is probably a consequence of three aspects of the problem which we have observed in analyses of endogenous viral loci in over 500 individual chickens of more than 30 breeds (ref. 3 and J. Wyban, J. Fang and S.M.A., in preparation). First, there is a large multiplicity of loci in the total population of chickens; second, individual chickens usually possess several loci (J. Wyban, J. Fang and S.M.A., in preparation); third, the laboratory flocks of white Leghorns all contain locus *ev* 1 (ref. 2), an observation which may reflect the derivation of this breed from a limited gene pool. Given these three factors, the production, from random matings, of a chicken free of endogenous virus would be a rare event. If endogenous viral genes are indeed non-essential, why are they so prevalent in the population? It has been proposed that endogenous viral genes have arisen as a result of evolution of cellular genes¹⁴. Alternatively, they may have arisen as a consequence of retrovirus infection of germ-line cells¹⁵. The large multiplicity of endogenous viral loci observed in the chicken population and the heterogeneity observed among individual chickens are consistent with the theory that the viral loci arose through independent germ-line infections. Integration of the proviral genome at random or at any one of a large number of sites^{16,17} would account for the observed large multiplicity of sites of residence of endogenous viral genes. In addition, differences in the genomes of the infecting virus and/or the occurrence of deletions, insertions or rearrangements in proviral DNA before integration would account for the heterogeneity observed among the endogenous viral genes themselves¹⁸.

Received 29 June; accepted 20 September 1979.

1. Todaro, G. J. *Cold Spring Harb. Conf. Cell Proliferation* **4**, 1169–1196 (1977).
2. Astrin, S. M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5941–5945 (1978).
3. Astrin, S. M. *et al. Cold Spring Harb. Symp. quant. Biol.* **44** (in the press).
4. Astrin, S. M. & Robinson, H. L. *J. Virol.* **31**, 420–425 (1979).
5. Groudine, M., Das, S., Neiman, P. & Weintraub, H. *Cell* **14**, 865–878 (1978).
6. Steeves, R. & Lilly, F. A. *Rev. Genet.* **11**, 277–296 (1977).
7. Rowe, W. P. *J. exp. Med.* **136**, 1272–1285 (1972).
8. Rowe, W. P. & Hartley, J. W. *J. exp. Med.* **136**, 1286–1301 (1972).
9. Frisby, D. P., Weiss, R. A., Roussel, M. & Stehelin, D. *Cell* **17**, 623–634 (1979).
10. Cohen, C. & Varmus, H. *Nature* **278**, 418–423 (1978).
11. Southern, E. M. *J. molec. Biol.* **98**, 502–517 (1975).
12. Roberts, R. J. *CRC crit. Rev. Biochem.* **4**, 123–164 (1976).
13. Padgett, T. G., Stubblefield, E. & Varmus, H. E. *Cell* **10**, 649–657 (1977).
14. Temin, H. N. *A. Rev. Genet.* **8**, 155–177 (1974).
15. Todaro, G. J., Benveniste, R. E., Callahan, R., Leiber, M. M. & Sheer, C. J. *Cold Spring Harb. Symp. quant. Biol.* **39**, 1159–1168 (1975).
16. Hughes, S. H. *et al. Cell* **15**, 1397–1410 (1978).

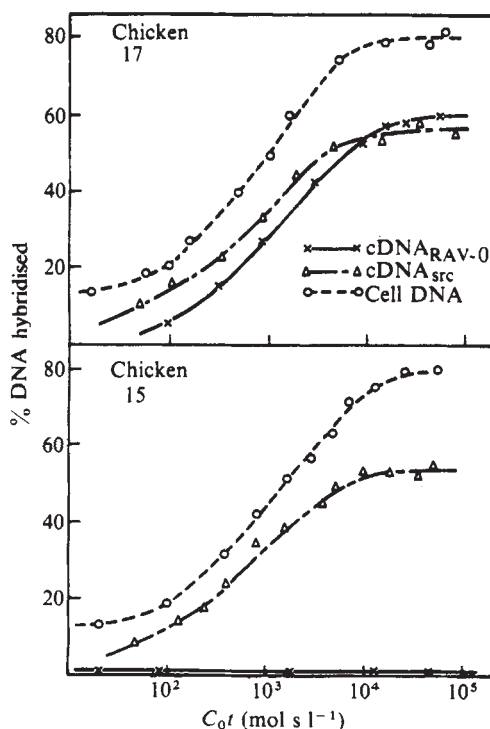


Fig. 2 Hybridisation of DNA of chicken no. 15 and chicken no. 17 to probes for total cell DNA, total RAV-0 sequences, and the cellular *src* genes (*src*). Probes were prepared as described by Hayward¹⁹. Re-association of cell DNA was monitored by addition of small amounts of 32 P-labelled cell DNA. Hybridisations were carried out in $4\times$ SSC using fragmented cell DNA at a concentration of 10 mg ml^{-1} and viral ^3H -labelled cDNA at 1 ng ml^{-1} (400 c.p.m. per reaction) in a total volume of 15–30 μl . Samples in sealed capillaries were heated to 100°C for 3 min and incubated at 68°C for various periods of time. Hybridisation was monitored by treatment with S_1 nuclease as described by Hayward and Hanafusa²¹. C_{ot} with values have been corrected to standard conditions of annealing as described by Britten and Kohne²². No corrections have been made for the differences in lengths of cell and probe DNAs. DNA lengths were approximately 150–200 nucleotides for unlabelled cell DNA, 150–200 nucleotides for 32 P-labelled cell DNA and 100–150 nucleotides for cDNAs.

17. Sabran, J. L. *et al.* *J. Virol.* **29**, 170-178 (1979).
18. Hayward, W. S., Braverman, S. & Astrin, S. M. *Cold Spring Harb. Symp. quant. Biol.* **44**, (in the press).
19. Hayward, W. S. *J. Virol.* **24**, 47-63 (1977).
20. Haseltine, W. A., Kleid, D. G., Panet, A., Rothenberg, E. & Baltimore, D. *J. molec. Biol.* **106**, 109-131 (1976).
21. Hayward, W. S. & Hanafusa, H. *J. Virol.* **15**, 1367-1377 (1975).
22. Britten, R. J. & Kohne, D. E. *Science* **161**, 529-540 (1968).

Structural similarities between Thy-1 antigen from rat brain and immunoglobulin

David G. Campbell*, Alan F. Williams*, Peter M. Bayley† & Kenneth B. M. Reid‡

* MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford

† National Institute for Medical Research, Mill Hill, London NW7

‡ MRC Immunochimistry Unit, Department of Biochemistry, University of Oxford, UK

The molecular basis of cellular interactions is poorly understood but it is commonly believed that direct interactions between membrane-borne ligands and receptors are important. Membrane molecules whose expression is regulated in differentiation are candidates for these roles. Functional assays for cellular interactions are of such complexity that the traditional biochemical methods of purifying a molecule by following a functional assay are not applicable. It can be argued that the best approach is first to identify cell-surface molecules and then to attempt to identify functions on the basis of their molecular characteristics or by using the molecules or antibodies to them to perturb functional systems¹. The Thy-1 (theta) antigen has been much studied because it is expressed in large amounts on some cell types but is absent from others. Thy-1 is probably the most abundant cell surface glycoprotein of rodent brain and thymus, yet its function is unknown (reviewed in ref. 2). On the basis of structural studies it was suggested that Thy-1 would be a candidate for displaying ligands for cell interactions and that the specific determinants involved might be carbohydrate³. We present here a preliminary report of further structural work dealing with the amino acid sequence and circular dichroism (CD) spectrum of rat brain Thy-1. The results suggest that this molecule has a structure resembling an Ig domain, which would not be consistent with an enzymatic function. The molecule probably exists to be recognised and the determinants involved could be protein or carbohydrate.

Thy-1 antigen has been purified from rat and mouse lymphoid cells^{4,5} and brain⁶⁻⁸. In all cases the molecule is a glycoprotein with an apparent molecular weight of about 25,000 determined by SDS-polyacrylamide electrophoresis. The correct molecular weight as determined by sedimentation equilibrium is 17,500 for rat brain Thy-1 and 18,700 for thymocyte Thy-1 (ref. 9). In both cases the polypeptide has a molecular weight of 12,500, the rest of the molecule being carbohydrate^{3,9}.

Thy-1 glycoprotein seems to have a hydrophobic portion because it binds one micelle of deoxycholate and forms a regular oligomer in the absence of detergent⁹. Also the molecule is heavily labelled when mouse thymocyte membranes are affinity labelled with a photoactivatable reagent which partitions into the lipid bilayer (M. J. Owen and M. J. Crumpton, personal communication).

Extensive amino acid sequence data for rat brain Thy-1 have been obtained by sequencing the peptides isolated after tryptic cleavage of succinylated Thy-1 (D.G.C., A.F.W. and K.B.M.R.,

manuscript in preparation). Overlaps were defined by sequencing peptides cleaved at Glu residues by means of staphylococcal V8 protease. The sequence begins with pyroglutamic acid (PCA) and contains 3 Cys and 1 Met as shown in Fig. 1. There is a disulphide bond from Cys 85 to Cys 9 or Cys 19 because reduction is required to release the two peptides obtained after CNBr cleavage at Met 84 (ref. 9). The disulphide bond thus includes 75 or 65 amino acid residues and this is similar to the residue number within intra-chain disulphide bonds of immunoglobulin (Ig) domains (55 to 75 residues¹⁰).

The sequence of 105 amino acids contains no obvious hydrophobic sequences of the type found in glycoporphin¹¹ and the only extended stretch of amino acids without charged groups is from residue 3 to 15 (Fig. 3). Thus the mechanism by which Thy-1 interacts with the membrane remains unknown. It could be that a small hydrophobic peptide is missing from the C-terminus because Arg 105 has only been identified from a tryptic peptide. However, the known molecular weight and amino acid composition are quite consistent with the sequence already obtained, and it is thus unlikely that a large number of extra residues will be found.

When the Thy-1 sequence was compared with sequences of the domains of Eu myeloma IgG, homologies were apparent. These were particularly evident at the sequences around the Cys residues which form the intra-chain disulphide bond of the Ig domains. This is shown in Fig. 2, where sequences around

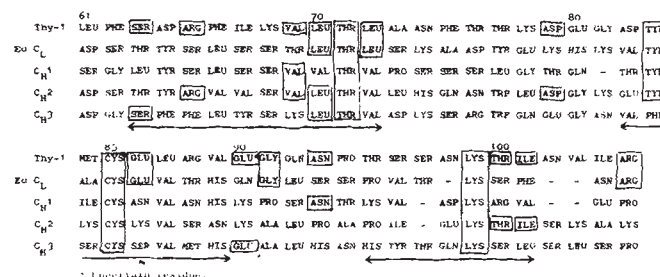


Fig. 2 Sequence comparisons between regions around Cys 85 of Thy-1 and the C-terminal Cys of the disulphide bridge in Ig domains. The sequences for the domains of Eu IgG are aligned as in ref. 12 for comparison of Ig domains with β_2 -microglobulin. Thy-1 Cys 85 is aligned with Cys 81 in the β_2 -microglobulin numbering. Positions are boxed where any Eu residue is identical to the appropriate Thy-1 residue. The arrows beneath the EuC_H3 sequences indicate the regions expected to be involved in β -sheet structures on the basis of the analysis of Beale and Feinstein¹⁴.

the C-terminal Cys of the Ig domain are compared with those around Cys 85 of Thy-1. The alignment of the Ig domain is that used by Peterson *et al.*¹² for comparison of β_2 -microglobulin with Ig and the Thy-1 sequence matches this alignment on both the N-terminal and C-terminal side of Thy-1 Cys 85 (the alignment with Thy-1 could be improved by altering the gaps in the Ig sequences in the region aligned with Thy-1 Lys 99). Most of the homologies occur where the Ig sequences are conserved, notably at residues in the regions aligned with Thr 71, Tyr 83 and Lys 99 of Thy-1. These sequences are in the regions which form the antiparallel β -sheet structure which is conserved in all Ig domains^{13,14} (arrows in Fig. 2). In comparison with the Eu IgG domains the direct homology is best with the C_L and C_H2 domain sequences where identity with Thy-1 occurs at 9 and 10 positions respectively out of 42 and 44. For β_2 -microglobulin compared over the same region as shown in Fig. 2 there is identity at 15 positions with C_L, 11 with C_H1 and C_H2 and 9 with C_H3 domain sequences¹².

A preliminary statistical analysis of the alignments shown in Fig. 2 has been done using the method of Moore and Goodman¹⁵ which is based on the number of residues aligned and the minimum number of mutations needed to make the sequences identical. Given these values the probability that the alignment is due to a random occurrence can be read from a published table¹⁵. For the alignments shown in Fig. 2 the probability of a



Fig. 1 Cys and Met amino acids in the sequence of Thy-1 glycoprotein.

reviews

Mediaeval scholarship

J. D. North

Science in the Middle Ages. Edited by D.C. Lindberg. Pp. 544. (Chicago University Press: Chicago and London, 1979.) £28; \$40.

JULIET'S opinion notwithstanding, there is a great deal in a name, and "science" means something very different now from what it meant even a century ago (when, for instance, Lockyer chose to call his journal *Nature*). It is not necessary, on the other hand, to pay too much attention to those paradoxes who would have us believe that there was *no* science (in the current sense of the word) in the middle ages. Even putting aside Aristotelian and other natural philosophy ("great mother of the sciences", as Francis Bacon called it), there is an abundance of material in works on astronomy, optics, medicine, biology, technology, statics and the science of motion, which would be classed as essentially scientific by the modern scientist. The temptation to focus on this positive achievement of the middle ages has in the past been too great for many scholars. The pendulum has recently swung perhaps a little too far in the other direction, and we are growing accustomed to oh-so-scholarly articles uniting the most disparate of themes, so that no-one would now raise an eyebrow, for instance, at "The influence of judicial combat on the theory of refraction", or "Monastic poverty and the law of free fall". The most conspicuous merit of the collection of essays edited by Professor Lindberg is that their authors, despite an occasional attempt to tack, usually steer a sensible middle course between the two extremes.

There is little doubt that much scientific work of originality and worth remains hidden in manuscript, and that some is lost for ever. Of all the historian's main tasks, the one which tends to suffer least from these difficulties is that of tracing mediaeval influences in later periods. Many important studies have been opened up in this retrospective way; and here the study of mediaeval theories of motion, leading to Galileo and the successes of the seventeenth century, deserves pride of place. The chapters by William Wallace (on the philosophical setting) and John Murdoch and Edith Sylla (specifically on the science of motion) make excellent use of writings by such as Duhem, Maier and Clagett. The chapter by Murdoch and Sylla is notable for its mastery of the material, evidence of long and painstaking study. It

is perhaps the best short introduction to the subject in any language. They stress the importance and pervasiveness of doctrines of motion to *all* mediaeval natural philosophy. Within their terms of reference, optics and statics were not considered as being strictly a part of natural philosophy, while astronomy is a difficult case, with one foot inside and the other outside the subjects. They discuss the mathematics of free fall, which managed to leave Aristotle on the sidelines, and the theory of impetus, which was hardly compatible with Aristotle; but they show how at every turn the books of Aristotle were, so to say, the intellectual driving force behind it all. The mathematical rules of the Mertonian (Oxford) and Parisian philosophers are remarkable enough, but moderately well known to scholars. What will perhaps come as a surprise to many readers is the importance they ascribe to the influence of the Church's doctrine of transubstantiation on the general theory of motion, so that "one of the two most prominent later mediaeval explanations of alteration, the so-called succession of forms theory, was originally taken seriously and worked out carefully as an explanation of the alteration and augmentation or diminution of the Eucharist". Another mediaeval tradition which might have been thought liable to lead to sterility but which is deemed influential, is that of the invention of sophisms within the context of logic. But all this activity was *secundum imaginationem*: it followed the creative imagination, and there was no question of any appeal to experiment.

The same was largely true of the science of optics, expertly discussed by the editor himself in a survey running from the ancient atomists to Descartes. Again the attack was on a broad front, making use of mathematical techniques (as of ray optics), but also taking in the physiology of the eye, the psychology of vision, and even theology. Here the key western texts were inspired not only by Aristotle, Euclid and Ptolemy, but by Islamic precursors such as Ibn al-Haitham and Avicenna. The editor is again himself responsible for a chapter on the transmission of Greek and Arabic learning. One can hardly complain about the brevity of the remarks on (for example) the work done in Castile under Alfonso X, as the chapter is so very full, tailing off only towards the end, where we might have had more about the *dissemination* of the translations done in Sicily, the Spanish

peninsula, and elsewhere. The bibliographical references in this chapter are invaluable.

The mediaeval science of weights, discussed in a chapter by Joseph Brown, shared with optics and theories of motion an uncertainty of aim and content. Broadly speaking, two paths were followed: there was the quasi-dynamical approach of an early follower of Aristotle, and there was the Euclidean and Archimedean tradition. The mediaeval synthesis of the two was hesitant and imperfect. If we look to a later chapter by James Weisheipl on the "nature, scope, and classification of the sciences", I think we can detect one of the contributory causes, namely the sheer eclecticism of the mediaeval scholar. He wanted to combine an encyclopaedic coverage of his subject matter with that dangerous kind of confidence that comes from recording all conceivable points of view. In his more pragmatically orientated thinking, he was in many ways more successful than when he chopped logic and philosophy in the schools. (Divinity poses an unresolvable problem.) As John of Salisbury said somewhat impatiently, more time had been spent on Porphyry's questions than the House of Caesar spent in winning and in ruling the Empire of the World. Chapters on technology and economic progress (Brian Stock), science and magic, that is, a pragmatics of desire (Bert Hansen), astrology (touched upon by several authors in passing), and medicine (Charles Talbot), all show that when the chips were down the mediaeval scholar could dispense with the dialectical skills he had learned at his university — or at least that he could hold them in reserve to explain his failures.

Mathematics (Michael Mahoney), the science of matter (Robert Multhauf), and the universities as an institutional setting (Pearl Kibre and Nancy Siraisi), are all covered in ways which help the book to achieve a remarkable degree of comprehensiveness. Jerry Stannard's is a short but entertaining sketch of natural history, "a complex amalgam of fact and fancy". The two remaining chapters are by Edward Grant (cosmology) and Olaf Pedersen (astronomy), and are largely complementary. The first gives an outline of the Aristotelian cosmology with which the extraordinarily sophisticated picture arrived at by the mathematical astronomers had to be reconciled, if only for the sake of good form. The astronomy chapter provides an important reminder —

with many well chosen illustrations of instruments — that the astronomer at least was not averse to making observations of the real world. But to what extent he and other mediaeval scholars were doing real

science is a question best answered by reading the collection as a whole. □

J. D. North is at the Filosofisch Instituut der Rijksuniversiteit, Groningen, The Netherlands.

Formal theory of ecosystems

Theoretical Systems Ecology. Edited by E. Halfon. Pp. 516. (Academic: New York and London, 1979.) £43; £27.95.

THE opening sentence of the preface to this book claims that systems ecology is having a large impact upon all aspects of environmental research, and that the systems approach, with its body of concepts and techniques, has broadened the ecologists' perspectives and attracted students into ecology from other disciplines. The preface goes on to suggest, however, that there has been a lack of communication between theoreticians and modellers and field ecologists, and that the purpose of this book is to try to bridge the gap in communication.

Is it true that systems ecology, or the application of systems theory to ecology, is having an impact on environmental research? Many ecologists would disagree with the statement, and there are as yet relatively few examples of practical action on environmental problems having been taken as a result of studies of systems ecology. There is certainly much understanding of the ways in which

organisms respond to changes in their environment, and some of this modelling is based on concepts derived from studies of control systems. I suspect, however, that one of the reasons for the lack of communication between theoreticians and field ecologists is that the dependence of ecological models on systems theory is less than is believed by the theoreticians. Much of the modelling still based on statistical concepts developed even earlier than those of the systems analysts.

This volume contains 20 chapters written by 28 contributors, and divided into four main sections. Part I discusses some fundamental system problems, particularly the aggregation problem and its relation to sampling. Part II includes information on modelling approaches and philosophy, with emphasis on model structure and model properties. Part III introduces methodologies and computer techniques of system identification. Part IV contains studies on model analysis, principally of structural properties such as stability, flow analysis and general systems properties.

Undoubtedly, the book contains original contributions in theoretical systems ecology which may be important as a basis for future developments. As most of the chapters are almost unintelligible to anyone not already well versed in systems

theory, however, the book does little, if anything to bridge the gap between theoreticians and practical ecologists. Indeed, until theoreticians can learn to state their conclusions in simple and unambiguous plain language, it is doubtful if systems ecology will really have the impact that systems analysts claim for it. At present, those of us who are attracted by this approach to ecology too often mistake our models for the real world, and are content to leave our description of these systems in the mathematical terms for which they were derived. Those who have to use our research find it difficult to translate from the mathematics to the plain statements of every-day usage.

One of the problems that need to be solved is terminology. The terms used in this book are claimed to be as uniform as possible, but different names are frequently used for the same concept or the same name for different concepts. The problem is said to be typical not only of systems ecology but also of general systems theory. This confusion seems to derive from the fact that the contributors follow the terminology of quite different theories of general systems, and the concepts of these theories do not often overlap. An early priority for theoreticians, therefore, would seem to be the resolution of these inconsistencies, and the development of a formal theory of ecosystems as a working definition for ecological research.

J.N.R. Jeffers

J.N.R. Jeffers is Director of the Institute of Terrestrial Ecology, Natural Environment Research Council, Grange-over-Sands, Cumbria, UK.

Primary energy supply prospects

World Energy: Looking Ahead to 2020. Report by the Conservation Commission of the World Energy Conference. (IPC Science and Technology Press: Guildford, UK, 1978.) £12.50; \$32.50.

THE objectives of the Conservation Commission were to evaluate, for the period 1985-2020, future primary energy supply prospects and to identify possible courses of action to overcome potential shortfalls in energy availability, particularly the impending decline of oil and gas supplies. The report is based on the work of a series of specialist study groups, dominated by OECD-centred experts.

The Commission notes that for industrialised nations there seems to be a decreasing requirement for additional energy supplies with increasing economic growth; by 2020, it seems that the energy coefficient might decline to one half its current average level in these countries (but it is unclear whether measurements are made in primary, delivered or final useful energy terms). In

the case of developing countries, however, the incremental ratio of energy demand to economic growth will continue to increase.

Somewhat surprisingly, given the upsurge in public opposition to virtually any major energy siting decision (for example, refineries, coal mines and nuclear stations) and evidence of rising real capital costs, the Commission argues that "the energy supply which could be attained in the next few decades is easier to predict than is the demand for energy, because it is determined primarily by technology". The Commission forecasts that the share of conventional crude oil in total world primary energy supplies will decline from 40% at present to about 10% by 2020, with natural gas then contributing 20%, non-conventional petroleum (for example, tar sands, shales, geopressure zone gas) about 5%, coal 25%, and renewable sources 10%, with the balance, 30%, being provided by nuclear energy from fission.

Total primary energy demand is estimated to reach three times today's level of 9×10^9 metric tons of coal equivalent by 2020, involving major efforts in all energy resource areas. In particular, it implies tripling coal production, "doubling nuclear power plant capacity every six

years" and substantial growth in solar energy.

The report envisages that about 30% of the energy that would otherwise have been needed by 2020 could be saved through technological improvements in conversion and utilisation. Further conservation, about 17%, is considered feasible through structural changes in the more mature economies, including saturation in car transportation and domestic energy demand and reduced utilisation by the energy-intensive industries.

The study is a valuable contribution to the literature, even though it omits sufficient discussion of issues such as public opposition, potential climatic impacts of increased energy consumption, geopolitical stresses likely to be prompted by international disparities in energy resource endowments, criteria for allocation of R and D expenditure, and more speculative and normative assessments of alternative world futures.

John Chesshire

John Chesshire is a Fellow of the Science Policy Research Unit, University of Sussex, Brighton, UK, and a member of the Unit's Energy Programme Group.

Protein phosphorylation

Protein Phosphorylation. By M. Weller. Pp. 557. (Academic/Pion: London, 1979.) £29.

ALTHOUGH proteins such as casein and phosphatase have been known for many years to contain covalently bound phosphorus, it is only since the discovery of enzyme regulation by phosphorylation-dephosphorylation in 1956 by Krebs and Fischer, and the discovery of cyclic AMP-dependent protein kinase in 1968 by Walsh, Perkins and Krebs that interest in protein phosphorylation has increased dramatically. The number of enzymes known to be regulated by phosphorylation-dephosphorylation has increased from 4 in 1969 to about 25 today, and the number of phosphoproteins (as opposed to enzymes) that have been identified exceeds 100. These include such familiar proteins as fibrinogen, ovalbumin and pepsin, in which the role of phosphorylation is still unknown.

The past five years have established protein phosphorylation as the major general mechanism by which intracellular events in mammalian tissues are controlled by external physiological stimuli and research in this area may well account for 5–10% of current biochemical and pharmacological research. Protein phosphorylation is not only implicated in enzyme regulation, but in the control of secretion, ion transport, protein turnover, chromosome condensation and viral transformation. Protein-bound phosphate also plays an important role in the binding of divalent cations such as iron and calcium, and in bone formation. Furthermore, many enzymes form phosphorylated reaction intermediates as part of their catalytic mechanism. Six different amino acid side-chains (serine, threonine, histidine, lysine, glutamic acid and aspartic acid) are known to be phosphorylated.

Dr Weller is to be congratulated on being the first person to attempt the monumental task of writing a comprehensive book on all these different aspects of protein phosphorylation. The book is readable, very comprehensive in its subject matter and remarkably free of gross factual errors and printing mistakes. It comprises 13 chapters and covers work published up to about the beginning of 1977. Nearly 3,600 references are listed at the end of the book.

As always the major problem of writing a book on a topic that is progressing rapidly, is that large sections are already out of date on the day of publication. Indeed, after reading this book one can see how much progress has taken place in some areas over the past two or three years. The chapters dealing with cyclic AMP- and cyclic GMP-dependent protein kinases and

the molecular specificity of these enzymes is already well out of date. The sections on enzymes such as pyruvate kinase, pyruvate dehydrogenase, acetyl-CoA carboxylase and initiation factors of protein synthesis which merit only a few lines in the book, would have to be expanded considerably today. Unfortunately the calcium dependent regulator protein story, which has revolutionised our understanding of the role of calcium in cellular control, and the interrelationship between cyclic AMP and calcium ions, broke just too late to get included. Naturally, enzyme systems discovered since this book was written, such as those involved in triglyceride, steroid, neurotransmitter and hormone synthesis are not included. Perhaps the major criticism that could be levelled at the

book is that it is too much a catalogue of facts, and more could have been done to relate protein phosphorylation to the integrated metabolism of the whole cell. On the other hand, chapters dealing with topics that are no longer such active research areas, such as the caseins and phosphatases, will remain complete and accurate accounts of work in these areas for many years.

However, there is no doubt in my mind that this book is a remarkable achievement and that it is an extremely valuable background reference book for all people engaged in research in this area.

Philip Cohen

Philip Cohen is Reader in Biochemistry at the University of Dundee, UK.

Sensory mechanisms of the spinal cord

Sensory Mechanisms of the Spinal Cord. By W.D. Willis and R.E. Coggeshall. Pp. 485. (Plenum: New York, 1978.) \$35. Available in the UK, Africa and the Middle East from Wiley (Chichester, UK) at £20.

THIS book will bring some coherence and stability to a confused subject. The confusion is partly inherent in the subject itself: inevitably the details of physiological mechanism, and, for the most part, micro-anatomical structure, come from experiments on a variety of higher animals, and any relevance to sensory mechanisms in man has to be established with the greatest caution. This kind of experimental evidence has appeared explosively in the past 15 years or so and the degree of caution has left much to be desired. So, although this is a detailed and wide-ranging monograph, it is a pleasure to find that it begins with a cautionary list of the types of general theory which have been advanced at various stages to bridge this gap and points out where these have been flawed by experimental error, unjustified assumptions, or plain mental confusion. The various 'dual' theories about somatic sensation are examined in this way and all found wanting. And an elementary but important semantic point is made that the word "modality" in sensation is a subjective and not objective term — one which defines quality in what we feel, and not, as many continue to treat it to the great confusion of others, a term describing an instrumentally measurable attribute of a stimulus. Similarly the book ends with a serious attempt to define, where evidence allows it, the sense-organs, tracts and nuclei which form the various channels on which the brain depends for its

information in different kinds (or modalities) of sensory experience.

The main bulk of the book is concerned with categorising and summarising recent evidence. Enough is said about sense-organs to form a sound basis for what follows; but the main detailed subject-matter is the anatomy and physiology of the sensory mechanisms of the spinal cord and medulla, that is, in general terms, the early stages of synaptic processing of incoming information and its channelling into a variety of new specialised paths. This is a field in which the authors and their close colleagues have a large personal experience and have made important contributions. The terminology and specialised techniques are sufficiently explained to make this quite easy reading. A good test of a comprehensive text like this is to use it. I used it to answer a variety of fairly esoteric questions to which I did not have a definitive answer and found its layout conducive to doing this quickly. The chapter titles and subheadings are reliable. The summaries are particularly helpful: each section has a short italicised summary and each chapter a set of concise conclusions. The bibliography is excellent.

The subject is advancing in full spate. This book is undoubtedly the best available text, indeed the only one of the present period, and it covers material published up to and including 1977. The form of the book is such as to allow updating and it is very much to be hoped that this will be done in due course. It is an expensive book for students, who would find the first and last chapters by themselves an excellent introduction to a subject which is potentially confusing and usually poorly dealt with in the general run of textbooks. The bulk of the book, however, is for reference or for the worker in the field. As such it is very much to be welcomed and not overpriced.

George Gordon

George Gordon is Reader in Sensory Physiology at the University of Oxford, and Research Fellow, Brasenose College, Oxford, UK.

Assessment of pesticides

The Use and Significance of Pesticides in the Environment. By F. McEwen and G. Stephenson. Pp.538. (Wiley: New York and Chichester, UK, 1979.) £16.75.

THIS book is based on lectures for a course that the authors have run for several years at the University of Guelph, and it is intended to give senior undergraduates a broad assessment of pesticides. The authors have achieved a high degree of detached assessment, based on the premise that although pesticides are virtually essential they also can and do present serious problems. On occasion this detachment almost becomes a fault, as both sides of some issues are presented in such a way that the authors' opinion is not immediately obvious. Students are unlikely to have the background of knowledge and experience to decide for themselves what reasonable assumptions can be drawn from a series of disjointed facts unless there is a fairly clear critical assessment of the issues involved.

The introductory chapter outlines the development of modern synthetic insecticides during and since the Second World War, the consequent problems of resistance to insecticides, the contamination of the environment by persistent insecticides, the effects on wildlife, possible hazards to human beings, and the possible role of integrated control. Several brief chapters then describe the types of issue raised by the use of pesticides, their use in public health, agriculture, forestry and 'rights-of-way', and for domestic purposes. No mention is made of industrial uses, particularly the treatment of wool and timber, which is causing much discussion in some parts of the world. A brief elementary but good chapter describes current testing procedures for the development of new pesticides: it should perhaps have referred to the current pressure for increasingly extensive and expensive toxicity tests, with the possible repercussions on the ability and willingness of chemical companies to develop new pesticides.

The major part of the book falls into two sections. The first gives brief accounts of the chemical, physical and biological properties of many individual pesticides, and the second describes the occurrence, movement and effects of pesticides in various parts of the environment — soil, water, air, food, birds, mammals and the biosphere.

This is a book that requires intelligent skimming, which would be helped if each chapter started with a list of its subdivisions. There is much material that may be useful for reference, but surely not all to be considered in detail. Examples are taken principally from North America, but

this does not altogether detract from the book's value for a British or European readership. For instance, the accounts of spraying against spruce budworm in the forests of New Brunswick, the consequent effects on salmon in the Miramichi River, and the effects of parathion on agricultural workers in California are all of general interest.

I would fault the book on details. The section that describes individual pesticides, despite its extensive coverage, is not too well integrated with the section on pesticides in the environment: residues of dichlobenil and BHC are mentioned, but the student will find no account of their chemistry and properties. The decline of peregrine falcons (*Falco peregrinus*) in Great Britain is attributed to eggshell thinning, instead of the acute toxicity of dieldrin poisoning. The authors do state that pesticides in water can be associated with particulate matter, but quote LC_{50} values for DDT of up to 2,400 p.p. 10^9 , whereas the best estimates for the maximum solubility of DDT in water are a little over 1 p.p. 10^9 . Similarly no comment is made on this aspect of Wurster's work on

the effects of DDT on phytoplankton at concentrations of 10–100 p.p. 10^9 .

On occasion too one would like the authors' opinions. The Delaney amendment in the USA, which prohibits the use on food of any pesticide that has been shown to be carcinogenic, is mentioned without any comment, although eighty pages later two and a half pages are devoted to testing for carcinogens. Again we are told, without comment, that "environmentalists" have criticised severely the use of dieldrin against fire ants. This is a programme of which those outside of North America may have heard but may lack detailed knowledge.

There is remarkably little about resistance — it is not listed in the index at all — nor about the wider ecological implications of pesticide use. To conclude, this is a book worth dipping into, although it will not suffice on its own for a broad assessment of pesticides. **F. Moriarty**

F. Moriarty is a Principal Scientific Officer at Monks Wood Experimental Station, Institute of Terrestrial Ecology, Abbots Ripton, UK.

Crystal structure determinations

Inorganic Molecular Dissymmetry. By Y. Saito. Pp. 167. (Springer: Berlin, Heidelberg and New York, 1979.) \$42.90; DM78.

PROFESSOR Yoshihiko Saito pioneered in 1955 the determination of the absolute stereochemical configuration of chiral transition metal coordination compounds by the anomalous X-ray scattering method, following the application of the technique by Bijvoet in 1951 to (+)-tartaric acid. The latter study showed that the arbitrary stereochemical convention of Emil Fischer, proposed in 1891, is in fact correct, while the former demonstrated that Werner's prototype dissymmetric complex, (+)-tris(ethylenediamine)cobalt(III), (+)-[Co(en) $_3$] $^{3+}$, has the morphology of a left-handed three-bladed propeller, later termed the Δ -configuration.

More recently Professor Saito has measured the electron-density distribution around the metal ion in transition metal complexes from the difference synthesis maps obtained through the use of automated X-ray diffractometers. In the case of [Co(en) $_3$] $^{3+}$, for example, it is found that the cobalt(III) ion is almost uncharged, in agreement with Pauling's electroneutrality principle, and that the

3d-electron density distribution is aspheric, with lobes located at the corners of a cube centred on the metal ion, while the electron density of the nitrogen non-bonding electron-pairs forming the coordinate bonds are somewhat displaced from the line joining the nitrogen atom to the metal ion.

His book, *Inorganic Molecular Dissymmetry*, provides a concise account of the principles underlying these studies and of their application in selected crystal-structure determinations, together with the theoretical basis and the practical articulation of conformational analysis applied to metal chelates, and of the investigation of d-electron optical activity in chiral transition metal complexes. The introduction to the book covers the types of isomerism encountered in metal complexes, and used by Werner in his structure theory, symmetry notation, and the specification of absolute configuration. The illustrations are clear and instructive, as are the mathematical appendices.

The book is directed towards a general and synthetic understanding of chiral molecules, and their unique property of optical activity, in the field of transition metal chemistry. The level of treatment is suited to graduate or advanced undergraduate teaching. For these roles, and for library reference, the book is strongly recommended.

S.F. Mason

S.F. Mason is Professor of Chemistry at King's College, University of London, UK.